PART I (OF TWO PARTS)

VOLUME 74 *August, 1956* NUMBER 2

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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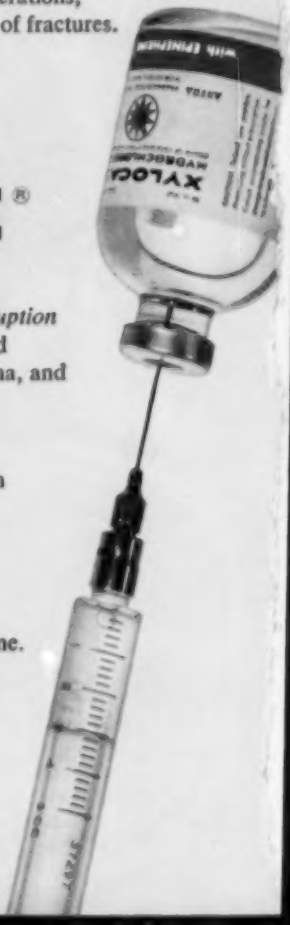

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*Southworth, J. L., and Dabbs, C. H.: Xylocaine: a superior agent for conduction anesthesia, Anesth. & Analg. *32*:159 (May-June) 1953.

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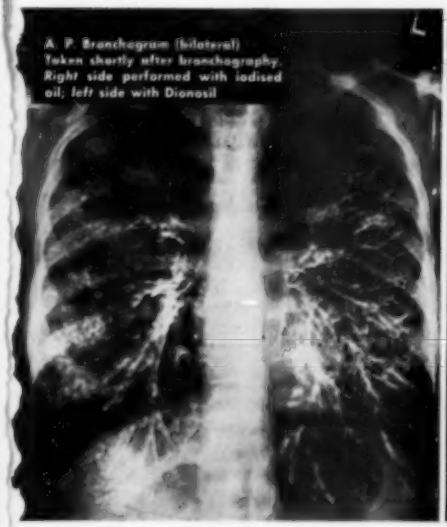

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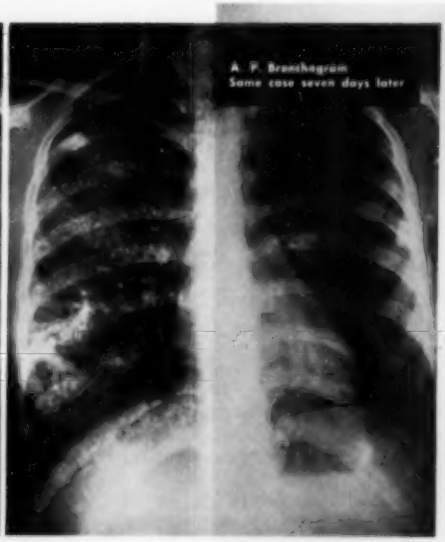
Right side performed with iodised oil; *left* side with Dionosil



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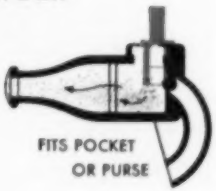

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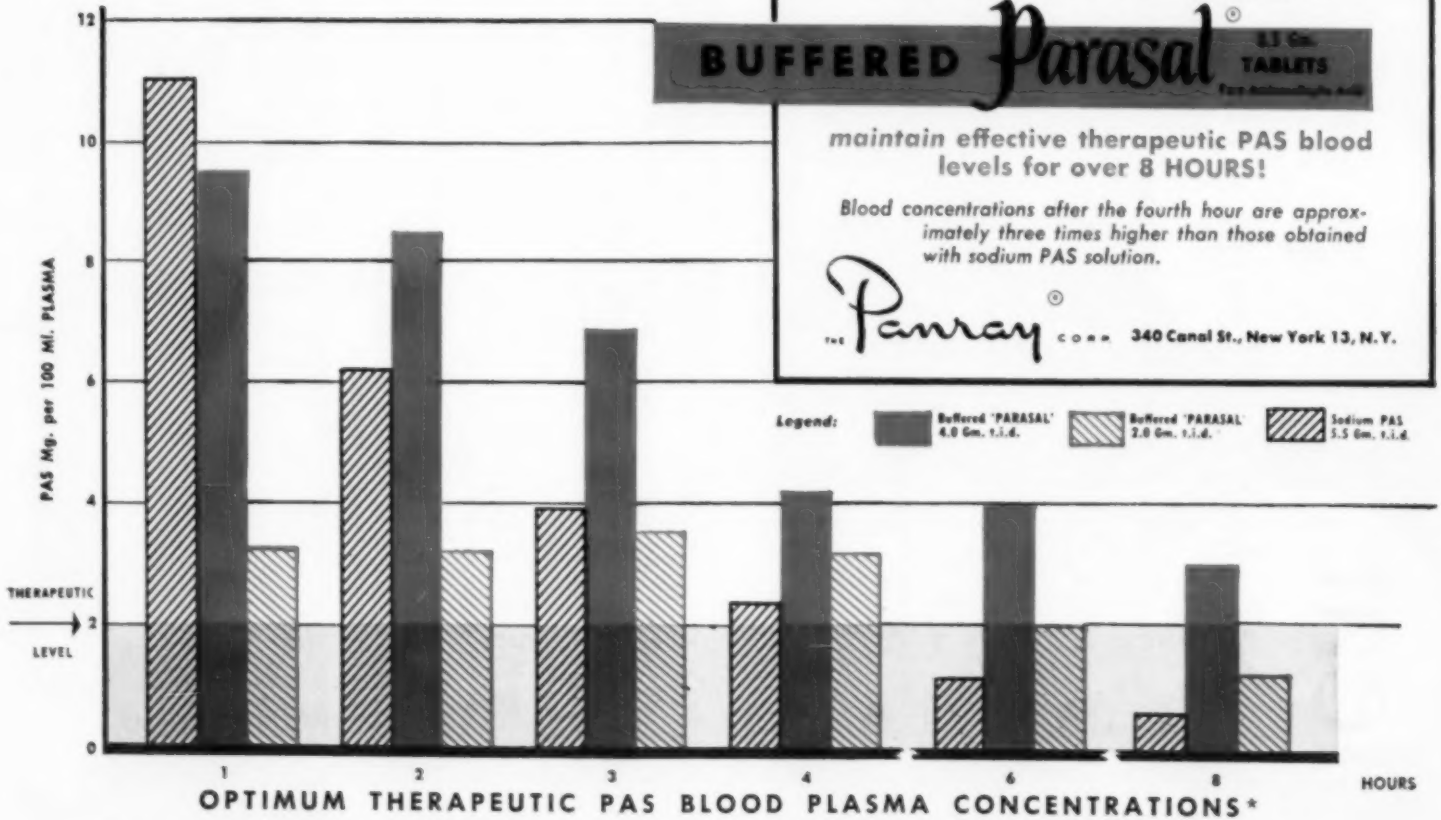

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Ref.: Deeb, E. N. & Vitagliano, G.R.: A. Rev. Tuber. & Pul. Dis.: 72,543-7 (Oct. '55)

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1. Shils, M. E.: Food and Nutrition Relating to Work and Environmental Stress, in Wohl, M. G., and Goodhart, R. S.: Modern Nutrition in Health and Disease, Philadelphia, Lea & Febiger, 1955, pp. 947-976.
2. Forbes, E. B., and Swift, R. W.: Associative Dynamic Effects of Protein, Carbohydrate and Fat, J. Nutrition *27*:453 (June) 1944.
3. Forbes, E. B.; Swift, R. W.; Marcy, R. W., and Davenport, M. T.: Protein Intake and Heat Production, J. Nutrition *28*:189 (Sept.) 1944.
4. Johnson, R. E.: Nutritional Standards for Men in Tropical Climates, Gastroenterology *1*:832 (Sept.) 1943.
5. Berg, C. P.: Utilization of Proteins, J. Agr. & Food Chem. *3*:575 (July) 1955.

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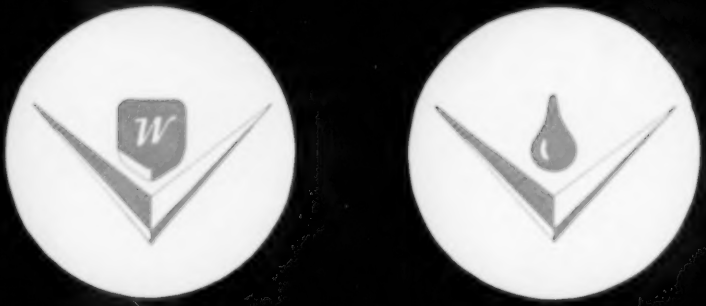

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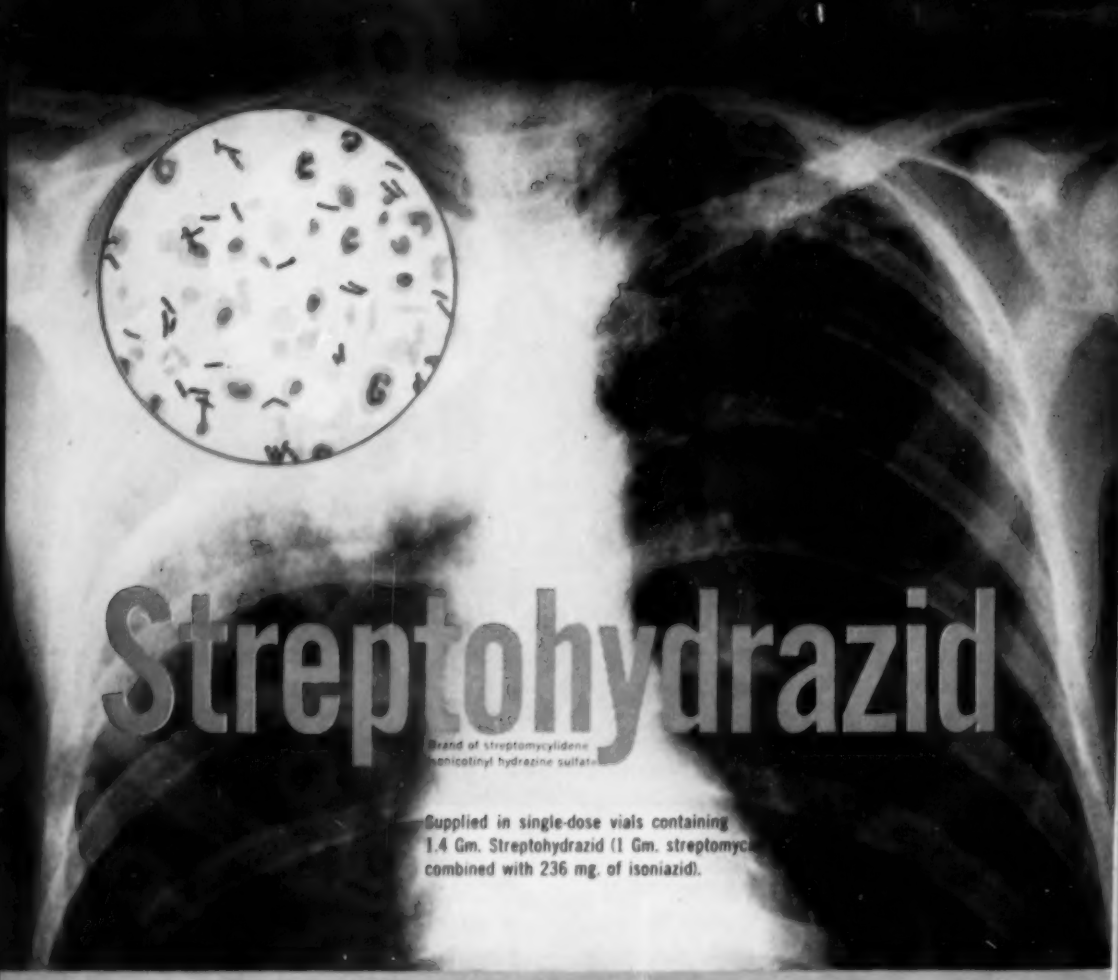

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1. Shubin, H., and Holtzen, C. A.
2. Morton, R. F.; LaCaille, R. A.; Gold, A., and Prigot, A.
3. Payne, H. M.; McKnight, H. V., and Harden, K. A.

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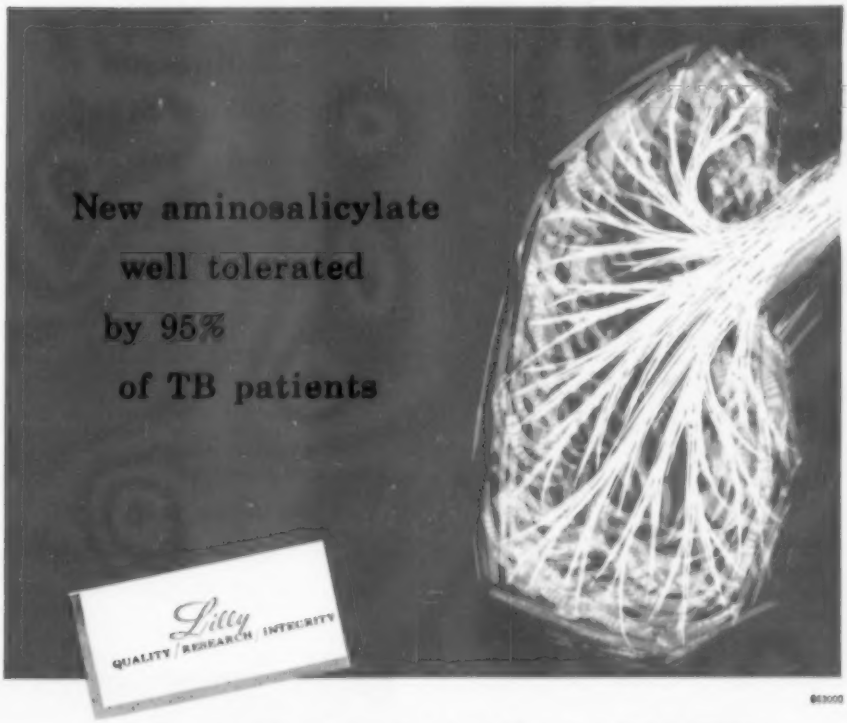

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*Chernish, S. M., and Peck, F. B., Jr.: Antibiotic Med., *1*:377, 1955.

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1. Pleasure, H.: in Chlorpromazine and Mental Health, Philadelphia, Lea & Febiger, 1955.

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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THE PROBLEM OF RESECTION SURGERY FOR PULMONARY TUBERCULOSIS IN THE NONINFECTIOUS PATIENT WITH PERSISTING CAVITARY DISEASE[1]

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INTRODUCTION

The resection of cavitary or noncavitary tuberculous lesions in patients receiving effective chemotherapy has been associated with a remarkably low surgical morbidity. In contrast, postoperative complications have occurred in nearly one quarter of the patients treated by pulmonary resection in whom the tuberculous infection was not controlled by antimicrobial therapy (1).

This report is a detailed study of 8 patients who underwent partial pulmonary resection for cavitary tuberculosis at a time when tubercle bacilli had been absent from the sputum for three months or longer.

The rather prompt occurrence of tuberculous reactivation in patients anticipated to be safe candidates for excisional surgery led to a careful examination of the surgical problem in the group of patients with cavitary tuberculosis in an apparently noninfectious state.

In a recent review of 212 previously untreated patients who had tubercle bacilli in the sputum and roentgenographic evidence of cavity when chemotherapy was started, Raleigh (2) found that 80 per cent had achieved noninfectious status after eight months or more of the chemotherapy. In this group, 84 (39.6 per cent) were found to have roentgenographic evidence of a persistent cavity at the six- to eight-month observation point although their sputum cultures had been negative for tubercle bacilli for the preceding three months or longer. These patients have been designated as the "noninfectious cavitary" group (3).

Early in our experience with this group, the surgical attitude was conservative because of the unknown incidence of relapse without surgery. The initial impression of a high risk of relapse in this particular group became more certain as the number of patients with residual annular shadows but no tubercle bacilli in the sputum continued to accumulate. For example, among 35 patients who were in the noninfectious cavitary status as they were completing their planned treatment, and who were not suitable candidates for surgery, 18 (51 per cent)

[1] From the Veterans Administration Hospital, Sunmount, New York.

had roentgenographic or bacteriologic evidence of tuberculous relapse. Of these 18 relapses, 8 occurred while the patients were still on their original chemotherapy. The remaining 10 relapses appeared after the chemotherapy had been discontinued or altered.

This observation was the reason for recommending surgical resection of the lesion in every patient when the presence of a cavity could not be excluded, even though tubercle bacilli had been absent from the sputum for several consecutive months. Moreover, in the similarly noninfectious patients with closed lesions (4), the absence of postsurgical tuberculous complications encouraged the employment of a similar variety of surgical procedures in this group with persistent cavities.

Confidence that a patient could avoid surgical morbidity because he met the prerequisites of certain roentgenographic and bacteriologic criteria was lessened by the fatal complication which ensued in the patient of the following case report:

Case Report

C. T., a 46-year old Negro male, had tuberculosis diagnosed by roentgenographic and bacteriologic examinations in May, 1950. He was treated with streptomycin-PAS for one year, after which he left the hospital against medical advice. Intermittent signs and symptoms, such as weight loss, hemoptysis, dyspnea, chest pain, and cough, persisted during the next two years. He was hospitalized at Sunmount in July, 1953. Re-treatment chemotherapy was begun with streptomycin, 1 gm. daily, and isoniazid, 300 mg. daily.

Tubercle bacilli had disappeared from the patient's sputum six months after admission to the hospital. Evidence of cavitary disease persisted by roentgenogram, however, so that, after four consecutive months of negative sputum cultures, a left thoracotomy was performed. The apico-posterior segments of the left upper lobe and a wedge from the lingula were resected. The patient had an average convalescence from the operation, but tubercle bacilli reappeared in the sputum. During the first three postoperative months, the patient failed to gain weight and had a moderately productive cough. The area of density in the operative site of the left upper lobe cleared slowly. One night three months following resection, he had a sudden pulmonary hemorrhage and died.

At post-mortem examination,[1] the area in which resection had been performed was found to be a pseudo-cavity filled with clotted blood and lined with tuberculous granulation tissue. It was bounded by parenchyma on two sides and the parietal chest wall on the third. At the apex of the defect the stumps of the apical-posterior segmental bronchi were found to contain unhealed tuberculosis. Erosion of the tuberculous process into an adjacent pulmonary vein was identified as the cause of the fatal hemorrhage.

Although this patient was considered to be in the "noninfectious cavitary" status at the time of resection, a sputum culture obtained in the month of operation yielded tubercle bacilli in the postoperative period. The patient's strain was moderately resistant to streptomycin and completely resistant to isoniazid. In addition, the culture from the resected tissue was positive for tubercle bacilli, with the same pattern of drug resistance. This case illustrates the following points:

> Tubercle bacilli, fully susceptible to isoniazid, just prior to "sputum "conversion," after four months of negative sputum cultures, revealed complete drug resist-

[1] Specimen examined by Dr. E. M. Medlar.

TABLE 1

POSTOPERATIVE REACTIVATION OF TUBERCULOSIS IN NONINFECTIOUS CAVITARY PATIENTS

Susceptibility of Tubercle Bacilli

Patient	Drug Regimen	Number of Months Treated	Sputum Last Positive Before Resection	Time of Sputum Conversion	Primary Resected Specimen	Postoperative Sputum
			months			
C.T.	SM 1 gm./day PAS 12 gm./day INH 300 mg./day	9	6	SM-R* PAS-S* INH-S	SM-M.R.* PAS-S INH-M.R.	SM-R PAS-R INH-R
F.F.	SM 0.5 gm./day PAS 12 gm./day INH 300 mg./day added between resistance	6 3	5	SM-R PAS-S INH-S	SM-M.R. PAS-M.R. INH-S	SM-M.R. PAS-M.R. INH-S
P.R.	SM 1 gm./day PAS 12 gm./day INH 150 mg./day added between resistance	6 4	4	SM-M.R. PAS-M.R.	SM-M.R. PAS-R INH-S	SM-M.R. PAS-R INH-S
A.L.	SM 1 gm./b.i.w. PAS 12 gm./day INH 300 mg./day added between resistance	5 1	4	SM-R PAS-S	SM-R PAS-S INH-S	SM-R PAS-S INH-S
D.O'D.	SM 1 gm./day PAS 12 gm./day	11	8	SM-S PAS-M.R.	SM-R PAS-R	SM-M.R. PAS-R

SM = streptomycin
INH = isoniazid
*S = susceptible
M.R. = moderately resistant (50 to 200 colonies in 15 γ streptomycin per ml; 10 γ PAS per ml., and 1 γ isoniazid per ml.)
R = resistant (200 colonies to confluent growth in 100 γ streptomycin and PAS per ml., and 5 γ isoniazid per ml.)

ance. This was true for the bacilli discharged in the sputum at the time of resection and in the resected tissue itself (table 1).

In the presence of slight microbial resistance to PAS (colonial growth) and increasing resistance to streptomycin, the addition of a third drug, isoniazid, was, in effect, giving this drug alone. The subsequent development of resistance to isoniazid might have been anticipated.

At the time of resection, the patient was actually discharging tubercle bacilli from an open cavity and was receiving no effective drug therapy. This was shown by the results of culture of the resected tissue and was, probably, the significant factor in the postoperative complication.

In the entire series of 147 patients with persistent cavities but no tubercle bacilli in the sputum, there have been no major tuberculous complications in patients whose strains of tubercle bacilli remained susceptible to the chemotherapy administered.

MATERIAL

Further review of this group of 147 patients disclosed 4 additional cases of postsurgical tuberculous reactivations (table 1). Each patient had a residual annular shadow and sputum cultures negative for tubercle bacilli for three months prior to resection. Again, each patient had a similar surgical procedure, namely, a segmental or bisegmental excision. Roentgenographic evidence of exudative disease at or near the resection site, plus the appearance of tubercle bacilli in the sputum, within two or three postoperative months was mute testimony of a chemotherapeutic and surgical failure.

Although secondary resection was not possible in the patient who died, lobectomy in 2 patients and resection of the superior division of the left upper lobe in the third were performed as secondary procedures, with subsequent arrest of the disease. The disease in the fifth patient was arrested by re-treatment with a new drug combination. Ultimate arrest of the tuberculosis was obtained, therefore, by a combination of surgery and chemotherapy in 4 of the 5 patients who had primary postresection reactivation.

In an effort to find a common denominator for these complications in what had heretofore been considered a uniformly safe group, a review was made of the complete series of patients with persistent cavities without tubercle bacilli in the sputum. Data were available on 147 patients who had undergone surgical treatment from January, 1953, to January, 1955, after they had attained the "noninfectious-cavitary" status. Particular attention was given to the pre- and postoperative bacteriologic findings. The presence of resistant or susceptible bacilli was noted in the last "positive" sputum or resected specimen.

RESULTS

It may be seen (table 2) that all combinations of drug susceptibility and resistance existed in the strains of tubercle bacilli recovered from the "preconversion" sputum and from the resected specimen. For example, a total of 11 patients, including both initially treated and re-treatment groups, had various degrees of resistance demonstrated in organisms recovered from resected tissues. Nevertheless, there had been no evidence of drug resistance in any of their sputum cultures.

TABLE 2

RESECTED NONINFECTIOUS CAVITARY PATIENTS

Comparison Preconversion Sputa with Resected Specimen

Original Treatment		Re-treatment	
Sputum Culture	Specimen Culture	Sputum Culture	Specimen Culture
Susceptible	Susceptible 10	Susceptible	Susceptible 3
	Resistant 5		Resistant 6
	Negative 18		Negative 17
Resistant	Susceptible 1	Resistant	Susceptible 3
	Resistant 2		Resistant 11
	Negative 9		Negative 19

Resistance defined as more than 50 colonies growth in third tube (concentration: streptomycin—15 γ/ml; PAS—10 γ/ml; isoniazid—1 γ/ml).

TABLE 3

CHANGE IN BACILLARY RESISTANCE DURING NONINFECTIOUS CAVITARY PERIOD

Total cases	147
Number with "positive" specimen cultures	59 (40%)
Comparison susceptibility	
Last positive sputum with specimen	39
Data incomplete for comparison	18

No Change (Still Susceptible)		Moderate Resistance		Resistant		Net Change Toward Resistance	
Streptomycin	23	Streptomycin	3	Streptomycin	8	Streptomycin	11 (32%)
PAS	29	PAS	2	PAS	7	PAS	9 (23.5%)
Isoniazid	10			Isoniazid	2	Isoniazid	2 (16%)

In those patients however, with tubercle bacilli resistant to one or more drugs in the sputum (before "conversion"), the operative specimen, when positive by culture or guinea pig inoculation, almost invariably disclosed drug-resistant tubercle bacilli.

Further study of this material revealed that tubercle bacilli were being recovered by culture or guinea pig inoculation from 40 per cent of the specimens (table 3). Of these 40 per cent, representing 57 patients, the data were sufficiently complete in only 39 to allow comparison of the preoperative susceptibility pattern with that found in the resected tissue. In these 39, further analysis was undertaken to determine if microbial resistance progressed *in vivo* while the patient was apparently in the noninfectious state.

Net change in resistance of the bacilli to streptomycin was found in 11 (32 per cent), in resistance to PAS in 9 (23.5 per cent), and to isoniazid in 2 (16 per cent) of the patient strains. The pattern of drug resistance was *always* toward greater degrees of resistance than was seen in any of the preoperative sputum cultures.

It is worthy of emphasis that the development of resistant tubercle bacilli during the period of the noninfectious state appeared in the minority of the group with persistent cavities (11 per cent). In the majority, when the resected specimen yielded tubercle bacilli, there was no essential change from the preoperative susceptibility pattern of the bacilli found in the sputum.

Because there is no apparent way for the surgeon to know at operation whether a patient will subsequently have bacilli recovered from the resected material, the preoperative susceptibility patterns of the "noninfectious-cavitary" group were studied with this question in mind. It was found (table 4) that, of 147 patients, 45 (30 per cent) had tubercle bacilli with partial or complete resistance to one or more drugs in the preoperative sputum culture. Thirteen patients had drug-resistant tubercle bacilli in both the preoperative sputum and the resected lesion.

Furnished with this information, it might well be suspected that a patient with some degree of drug resistance in the last sputum culture obtainable would have a 30 per cent chance to show viable bacilli in the resected specimen of lung.

TABLE 4

RESECTED NONINFECTIOUS CAVITARY PATIENTS

Development of Drug Resistance before Reversal of Infectiousness

Cases	Number Showing Resistance to One or More Drugs	Number with Resistant Bacilli in Pre-conversion Sputum and Specimen*
Original treatment 68	12 (17.5%)	2
Re-treatment 79	33 (41%)	11
Total 147	45 (30%)	13 (9%)

* Closed or open lesions

DISCUSSION

A surgeon may be unaware that he is resecting tissue which harbors drug-*resistant* tubercle bacilli. Nevertheless, it is believed that local excision (including segmental resection unless the lesion is very discrete) should be avoided in any patient whose chemotherapy might be of doubtful effectiveness because of the probability of drug resistance. Success of secondary lobectomy in those patients who had tuberculous reactivation at or near the site of previous resection suggests this might have been the initial procedure.

From a study of this "noninfectious-cavitary" group, a clinical pattern has evolved which has definite chemotherapeutic and surgical implications. A patient who stops discharging tubercle bacilli in the sputum during the sixth to eighth month of initial chemotherapy may exhibit a persistent open lesion by serial planigrams. If the patient is considered a surgical candiate, he may then have any variety of resection performed without fear of tuberculous complications. A similar patient, whose strain of tubercle bacilli shows slight to moderate resistance to one or two of the major drugs prior to the reversal of infectiousness, should be carefully evaluated by the clinician in the event that surgery becomes necessary. The decision to be made is whether a third drug should be added or, possibly, a change made to two new drugs. Particularly suspect is the patient who is receiving a course of re-treatment with antituberculous drugs. The development of microbial resistance is well known to proceed at a greater rate in the patient with repeated courses of chemotherapy.

With this type of case, the writer's practice is to bring it to clinical review at an earlier date than that of the patient who is receiving an initial course of chemotherapy. The intention is, if surgery is recommended, to operate while the patient still harbors drug-susceptible organisms. If the degree of drug control is suggestively uncertain because of possible bacillary resistance to one or more drugs in the last obtainable culture, the assumption is that this patient's open cavity may contain drug-resistant organisms. In this situation, the resection procedure is tailored accordingly.

It is not intended to imply that the bacteriologic data herein reported are unique to one institution. Other hospitals have reported similar cases in which the last obtainable sputum culture yielded drug-susceptible tubercle bacilli, yet subsequent resection yielded drug-resistant bacilli in the lesion (5).

The writer is not aware that any previous attempt has been made to correlate the incidence of post-resection tuberculous reactivations with the presence of drug-resistant bacilli in resected tissues. An effort has been made to find a clue in the preconversion drug-susceptibility pattern which would enable a prediction whether a lesion harbored resistant bacilli at the time of resection.

Such prediction is evidently difficult. The factors which influence the emergence of drug-resistant bacilli are multiple. Among these factors are the nature and distribution of disease, the particular combination of antituberculous drugs being used, the duration of drug therapy, and whether or not the patient is being treated for the first time or has received extensive chemotherapy previously.

In the present report the purpose has been to draw attention to the lack of bacteriologic uniformity in the so-called "noninfectious-cavitary" patient, despite the similarity of the clinical pattern of the members of this group.

Although in less than half of the patients with drug-resistant bacilli in resected specimens, such resistance was apparent in the "preconversion" sputum, this group at least may be regarded by the surgeon as potentially unsafe for local pulmonary excision. In the entire noninfectious-cavitary group, all of the immediate postoperative reactivations occurred in patients with drug-resistant bacilli in the tissues. In retrospect, the strains from these patients had shown equivalent or less resistance in the last culture obtainable before reaching the "noninfectious-cavitary" state (figure 1).

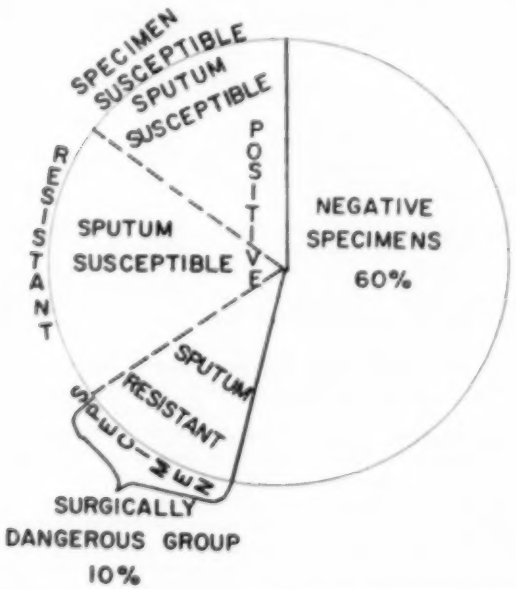

FIG. 1. Resected specimens in noninfectious cavitary patients

SUMMARY

The achievement of sputum "conversion" by initial or re-treatment course of chemotherapy does not necessarily guarantee sufficient drug control of tuberculosis of the lung for the purposes of pulmonary resection (6).

Various degrees of drug resistance to one or more drugs developed before reversal of infectiousness in 17.5 per cent of the patients who were receiving chemotherapy for the first time. Drug resistance was twice as frequent (41 per cent) in the re-treatment group. Those patients with partially resistant tubercle bacilli before reversal of infectiousness will show equivalent or greater resistance in the bacilli recovered from resected tissues.

In one third of the patients who showed some degree of bacillary resistance in the last obtainable culture of the sputum, tubercle bacilli were cultured from the resected specimen.

The "noninfectious-cavitary" group is not uniform from the surgical standpoint. Among the one third of the group whose strains of tubercle bacilli showed some drug resistance before reversal of infectiousness, there is a dangerous 10 per cent who actually have open cavities with drug-*resistant* tubercle bacilli when operated upon. In this 10 per cent with uncertain drug control, limited excisions (such as segmental or wedge resections), in the experience of the writers, have resulted in prompt tuberculous reactivation in the operative site.

While a variety of resections have been safely carried out in 90 per cent of the "noninfectious-cavitary" group, in this dangerous or unpredictable 10 per cent primary lobectomy should be performed. This policy of tailoring the surgery to fit the anticipated bacteriologic findings in the lesions has proved most satisfactory.

SUMARIO

El Problema de la Cirugía Reseccional por Tuberculosis en el Enfermo Negativo Abierto

La obtención de viraje del esputo con la quimioterapia primaria o repetida no garantiza forzosamente dominio eficaz con las drogas para los fines de la cirugía en la resección pulmonar (6).

Aparecieron varios grados de fármacorresistencia a una o más drogas antes de virar la infecciosidad en 17.5 por ciento de los sujetos que recibieron tratamiento primario, pero esto fué dos veces más frecuente (41 por ciento) en el grupo re-tratado. Los enfermos que tienen bacilos tuberculosos parcialmente resistentes antes de invertirse la infecciosidad revelarán resistencia equivalente o mayor en los bacilos obtenidos de los tejidos resecados, si éstos resultan positivos al cultivo.

En la tercera parte de los enfermos que mostraron alguna resistencia bacilar en el esputo antes de virar la infecciosidad, se obtuvo un ejemplar "positivo."

El grupo "negativo" abierto no resulta uniforme desde el punto de vista quirúrgico. Entre la tercera parte del grupo cuyos microbios del esputo muestran fármacorrresistencia antes de invertirse la infecciosidad, habrá un 10 por ciento peligroso que se encuentra realmente en la fase *resistente* "negativa" abierta al ser operado. En este 10 por ciento con incierto dominio quimioterapéutico, las excisiones limitadas, tales como las resecciones segmentales o cuneiformes, han dado por resultado, según han observado los AA. mismos, rápida reactivación tuberculosa en el lado operado.

Aunque se han llevado a cabo inocuamente varias clases de resecciones en 90 por ciento del grupo "negativo" abierto, en el 10 por ciento peligroso o problemático, se ejecuta la

lobectomía primaria. Esta práctica de amoldar la cirugía a la bacteriología esperada en los tejidos ha dado la mayor satisfacción.

RESUME

Le problème de la chirurgie d'exérèse pour tuberculose chez le malade atteint de lésions ouvertes sans bacilles décelables

Le succès de la négativation du crachat par le traitement chimiothérapique initial ou par la répétition du traitement ne constitue pas nécessairement une garantie de son efficacité à controler l'affection quand il s'agit d'effectuer la résection pulmonaire (6).

Divers degrés de résistance à l'un ou à l'autre des agents thérapeutiques se sont manifestés avant la disparition de la phase contagieuse chez 17,5% des malades ayant subi la cure initiale, mais dans le groupe ayant été soumis à une nouvelle cure, la résistance était deux fois plus fréquente (41%). Chez les malades qui hébergaient des bacilles résistants avant de devenir abacillifères, on a observé une résistance équivalente, voire même plus élevée, chez les bacilles isolés à partir des tissus réséqués dont la culture était positive.

Dans un tiers des cas, où un certain degré de résistance bacillaire existait dans les cultures de crachats avant leur négativation, un spécimen "positif" fut obtenu.

Le groupe des malades abacillifères avec lésions ouvertes n'est pas uniforme du point de vue chirurgical. Parmi le tiers des malades de ce groupe, ceux dont les germes isolés dans les crachats présentent une résistance au médicament avant que la négativation des crachats soit obtenue, il y aura ces 10% dangereux qui sont au stade *résistant* négatif et porteurs de lésions ouvertes au moment de l'opération. Chez ces 10%, où la stabilisation de l'affection est douteuse, des résections limitées, telles que les résections segmentaires ou l'ablation de foyers ont, selon l'expérience des auteurs, entraîné la réactivation rapide de la tuberculose au siège de l'intervention chirurgicale.

Bien que des résections diverses aient été menées à bien chez 90% des malades du groupe à lésions ouvertes sans bacilles décelables, chez les 10% qui représentent le groupe dangereux des risques opératoires, la lobectomie primaire est effectuée. Cette politique de la chirurgie "faite sur mesure" adaptable à la bactériologie des tissus s'est montrée des plus satisfaisantes.

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EVALUATION OF 3,5-DIIODO-4-PYRIDONE N-ACETIC ACID (DIONOSIL®) AS A BRONCHOGRAPHIC AGENT[1]

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INTRODUCTION

The unquestioned value of information often obtained by bronchography has been hampered by the disadvantages of the bronchographic agent since iodized oil was first introduced by Sicard and Forestier in 1922 (1). Evaluation of several aqueous contrast media with an appreciation of their limitations has been adequately described (2–6). Within the past three years experience with aqueous and oily preparations of the n-propyl ester of 3,5-diiodo-4-pyridone N-acetic acid (Dionosil®) has been gained by many investigators (7–13).

It was the purpose of the present study to evaluate whether or not Dionosil might be substituted advantageously for the iodized oil previously used with no other change in technique employed.

As the study progressed, it was apparent that this agent could be used in patients with pulmonary tuberculosis, and information so obtained would be useful in the manner emphasized by others (14–18). Thus, the routine use of Dionosil in both tuberculous and nontuberculous chest disease has been studied.

METHODS AND MATERIALS

Thirty-one adult male patients were included in this study. In no case was bronchography performed within three weeks of an episode of pneumonia, temperature elevation, hemoptysis, or induction of pneumoperitoneum. Twelve patients were referred for bronchography from the tuberculosis service to evaluate the nature of the residuals of pulmonary tuberculosis or the surgical results of pneumonectomy or thoracoplasty. All patients with tuberculosis had what was considered to be stable disease. Nineteen nontuberculous patients were bronchographed because of unidentified pulmonary pathology; of these, 13 were suspected of having bronchiectasis.

Oily and watery suspensions of a bronchographic medium[2] whose radio-opacity is due to n-propyl ester of 3,5-diiodo-4-pyridone N-acetic acid (Dionosil) were used. The aqueous preparation of this agent is a 50 per cent suspension containing 30 per cent iodine with sodium carboxy-methyl cellulose as a viscosifying agent, sodium citrate as a buffer, sodium chloride to render the mixture isotonic, and benzyl alcohol as a bacteriostatic agent. The oily preparation is a 60 per cent suspension in pure Arachis oil containing 34 per cent iodine and having no cellulose base.

One side of the tracheobronchial tree was explored twenty-five times using the watery preparation, and one side was explored twenty-six times using the oily preparation. Each patient received barbiturate, atropine, and codeine preoperatively, and 5 to 10 ml. of 10 per cent cocaine to obtain topical anesthesia of the nasotracheobronchial tree. Sensitivity tests for the agents were not performed. The medium was instilled via a soft nasotracheal tube and gravity filling of the necessary segments without use of special catheters was ob-

[1] From the Department of Medicine, Baylor University College of Medicine, and Veterans Administration Hospital, Houston, Texas.

[2] Supplied in part through the courtesy of the Picker X-ray Corporation.

tained by appropriate positioning of the patient. In each position, 2 to 4 ml. of the medium were introduced through the nasotracheal tube. The patients were fluoroscoped to determine adequacy of filling and to obtain spot films. Recumbent and upright films were taken as well as 24-hour follow-up films. Following the removal of the nasotracheal tube, many of the patients were encouraged to cough vigorously, and immediately thereafter postero-anterior films were obtained to evaluate any undesirable effects of cough.

For two hours following bronchography the patients were instructed to remain in the prone position, to take nothing by mouth, and to cough and expectorate frequently and vigorously. Prophylactic antimicrobial drugs were not used in any of the cases.

RESULTS

Fifty-one unilateral bronchograms were obtained from the thirty-one patients. Eleven of the twenty-five sides explored (44 per cent) with aqueous Dionosil were considered unsatisfactory due to inadequate filling. Four of the sides (15 per cent) explored with oily Dionosil were considered inadequate. No significant difference between right and left lung was noted in the adequacy of filling. Of the thirty-six sides considered to have been adequately filled, fifteen showed roentgenographic evidence of pulmonary abnormalities.

Films taken in the upright position showed the medium to be applied as a thin even coating over the mucosal surfaces extending down to the smaller bronchi (figure 2*B*). Recumbent films showed more opaque homogeneous density than did films taken with the patient in an upright position (figure 2*A* and *C*).

Instructing the patient to cough vigorously and examining the roentgenograms taken after forced cough revealed that marked coughing did not obscure detail (figures 2*B* and 4*B*).

Alveolar filling was noted in two instances, and in both cases the oily medium had been used. Despite this finding, all segments could be visualized with little loss of detail.

Any desired type of roentgenogram can be obtained after the medium has been instilled. The quality of the spot films was equivalent to the quality of the corresponding larger films (figure 1*B*). Lateral films may be made with the patient recumbent (figure 2*C*) or upright (figure 4*B*). Anteroposterior (figure 3*B*) or lateral (figure 3*C*) laminagrams may be obtained with no necessity to hurry. One segment or one lobe may be explored by use of proper positioning of the patient (figure 3*A*).

Twenty-four-hour follow-up films showed 90 to 100 per cent clearing of the medium in all sides explored with aqueous Dionosil. Fifteen of the twenty-six sides studied using oily Dionosil showed a similar degree of clearing, and the remaining eleven sides showed 70 to 90 per cent of clearing in the same period. When significant retention occurred, the medium tended to remain in the areas of greatest bronchial change (figures 1*C*, 2*D*, and 4*C*).

The procedure was well tolerated by all patients and in no instance was it necessary to interrupt the procedure or to give additional or increased amounts of preoperative or anesthetic drugs. No complications or toxicity were observed.

DISCUSSION

For the purpose of evaluation of Dionosil as a bronchographic agent, typical cases were selected using standard methods and equipment. The adaptability of

this new medium was noted from the onset of the study. The preparations are tasteless and well tolerated by the patients. The viscosity of the medium at room temperature enabled its use without special preparation. The suspension tended to settle out with long standing, and shaking was necessary to restore homogeneity. The medium was easily drawn into syringes, and sticking of the syringes or delay in instillation was not encountered. Clearing from the tracheobronchial tree was not so rapid as to necessitate acceleration of the bronchographic procedure, and complete mapping of the pulmonary segments was thus possible. The opacity of the medium allowed easy detection of accidental spillage on equipment or patient.

No complications were encountered during bronchography. An occasional cough could be easily controlled through patient cooperation, and in no instance were films unobtainable because of cough. The danger of using preparations of high viscosity, with their obstructive effect, has been pointed out by Norris and Stauffer (4), but none of the patients in the present series showed such an effect, although pre-existing increased resistance to air flow was present in many of the cases. The cycle of irritability leading to increased anesthesia mentioned in the early reports which, in turn, leads to increased danger was never encountered.

Oily Dionosil gave more satisfactory filling than did aqueous Dionosil. The finding of 15 per cent unsatisfactory filling with the oily Dionosil compares favorably with the 16 per cent unsatisfactory filling with iodized oil, and the 33 per cent unsatisfactory filling with the water-soluble agents as reported by Peck and associates (5). Whether or not deep breathing would lead to more satisfactory filling was not critically evaluated, although the value of such manipulation has been reviewed by Priviteri (19).

It was observed that much of the medium was cleared from the tracheobronchial tree by cough and expectoration. A smaller but significant amount was swallowed, but symptomatic gastrointestinal disturbances were not observed. The remaining material is thought to be hydrolyzed with the resulting alcohol metabolized and the remainder excreted in the urine as 3,5 diiodo-pyridone-N-acetate, without the liberation of iodide or iodine (20). Skin rash, coryza, temperature elevations, pulmonary complications, or spread of the tuberculous process were not observed in the postbronchographic period. Thus, Dionosil appears to be a safe bronchographic medium when used on patients with nontuberculous lesions or with controlled, stable, pulmonary tuberculous lesions (17).

Gudbjerg (21), in his description of oily and watery Dionosil, states, "Introduction of these media does not make a big step forward. Both cause greater local irritation than iodized oil and their contrast effect is distinctly inferior." The present writers have not found the material to be as irritating as iodized oil and have produced equivalent roentgenograms while avoiding the potential dangers of the combined iodized oil and sulfadiazine which Gudbjerg advocates.

Increasing pathologic evidence demonstrates that bronchographic agents may be a potential source of danger and forms the basis for advocating segmental bronchography (22). Felton (23) has shown that 26 per cent of those patients

with residual iodized oil gave histologic evidence of focal, peripheral foreign body granulomas surrounding the oil droplets. Contrasting studies with Dionosil by Cummins and Silver (11) demonstrated oil in the alveoli and alveolar walls in five pathologic specimens, but no granulomas were observed.

Holden and Crone (10) report that specimens obtained from six pneumonectomies five to twenty days after oily Dionosil had been used showed no free oil, and there was no evidence of local reaction except simple phagocytosis. Norris and Stauffer (12) studied five patients following aqueous Dionosil and noted only a peribronchial cellular response consisting chiefly of lymphocytes, plasma cells, and macrophages.

Thus, there has been no evidence yet presented of damage to the lungs by oily Dionosil. Because it contains no cellulose-like material, it is free of the potential dangers of a high molecular weight carbohydrate component. Nevertheless, it seems very important to recover the material as quickly as is practicable following bronchography, so that the medium is in contact with the bronchi for as short a time as possible to minimize possible complications.

Bronchography may be disappointing in outlining cavities, as pointed out by Holden and Crone (10), but other essential and confirmatory information may be obtained with only a small percentage of misleading information, as studies by Shaw and associates (14) have shown. Bronchographic information may allow more precise anatomic localization of lesions; may decrease the number of failures following collapse procedures, as noted by Marchese and co-workers (17); may permit better evaluation of bronchiectatic changes which have been shown to be present in more than one-half of the patients with tuberculosis (16); may give an indication whether bronchiectatic changes are of tuberculous or nontuberculous origin (18); and may provide information regarding the nature of the residual stump following resectional surgery. Complete preoperative mapping may be a valuable adjunct to lung palpation during surgery to determine the extent of diseased lung and to differentiate normal from abnormal lung. With Dionosil, resectional surgery need not be delayed because of slow clearing following bronchography.

SUMMARY

The routine use of Dionosil® in both tuberculous and nontuberculous chest diseases was studied. Thirty-one cases were selected and standard bronchographic methods and equipment were used.

No complications attributable to bronchography were encountered.

The oily preparation gave more satisfactory filling than did the aqueous suspension, and the ratio of satisfactory films was equal to that obtained by using iodized oil.

Clearing of the medium occurred in most cases within twenty-four hours. Cough seemed to aid in clearing without giving alveolar filling.

Oily Dionosil is a safe, well-tolerated bronchographic medium when used in patients with nontuberculous lesions or with controlled, stable, pulmonary tuberculous lesions.

SUMARIO

Justipreciación del Dionosil® como Agente Broncográfico

El uso sistemático del Dionosil en las afecciones torácicas, tanto tuberculosas como no tuberculosas, fué el objeto de este estudio. Seleccionados 31 casos, se usaron en ellos métodos e instrumental broncográficos aceptados.

No se observaron complicaciones imputables a la broncografía.

La preparación oleosa obtuvo henchimiento más satisfactorio que la suspensión acuosa, siendo la proporción de películas satisfactorias igual a la obtenida con el uso de aceite yodado.

La desaparición del medio tuvo lugar en la mayoría de los casos en término de veinticuatro horas. La tos pareció ayudar en el despejo sin provocar henchimiento alveolar.

El Dionosil oleoso es un medio broncográfico inocuo y bien tolerado cuando se emplea en enfermos que tienen lesiones no tuberculosas o lesiones tuberculosas pulmonares estabilizadas y bajo gobierno.

RESUME

Evaluation du Dionosil® comme agent de bronchographie

L'usage routinier de Dionosil dans les affections thoraciques tuberculeuses ou non tuberculeuses a été étudié. Trente et un cas ont été sélectionnés et un équipement et des méthodes bronchographiques standard ont été utilisés.

Il n'a été observé aucune complication imputable à la bronchographie.

Les préparations huileuses ont procuré un remplissage plus satisfaisant que la suspension aqueuse et la proportion de clichés satisfaisants était égale à celle obtenue avec l'huile iodée.

La disparition du milieu opaque s'est produite dans la plupart des cas dans les vingt-quatre heures. La toux paraissait favoriser l'évacuation sans entraîner le remplissage alvéolaire.

Le Dionosil huileux est un milieu bien toléré en bronchographie quand il est utilisé chez des malades sans lésion tuberculeuse ou dont les lésions pulmonaires sont contrôlées et stabilisées.

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Figures 1–4 follow

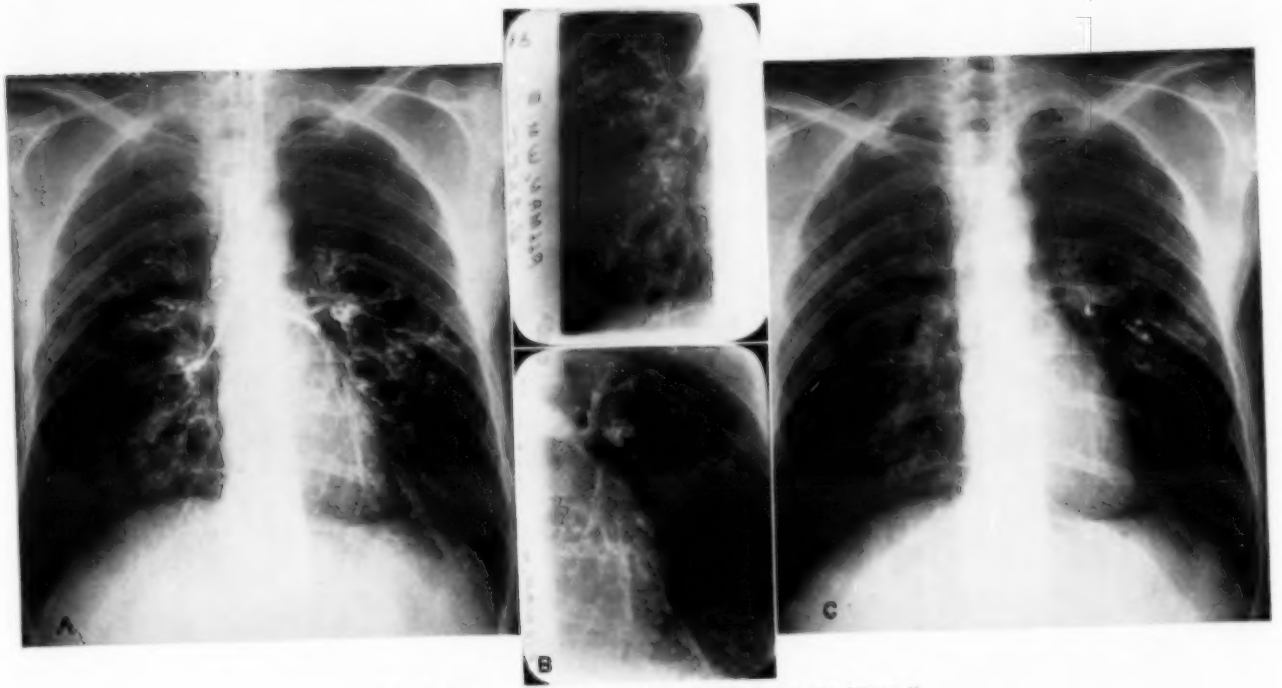

FIG. 1. Bilateral bronchography using 18 ml. of oily Dionosil.

A. Bronchogram shows presence of bilateral bronchiectasis.
B. Quality of spot films which can be obtained with oily Dionosil is shown.
C. Film shows amount of clearing in twenty-four hours despite the presence of bronchiectasis.

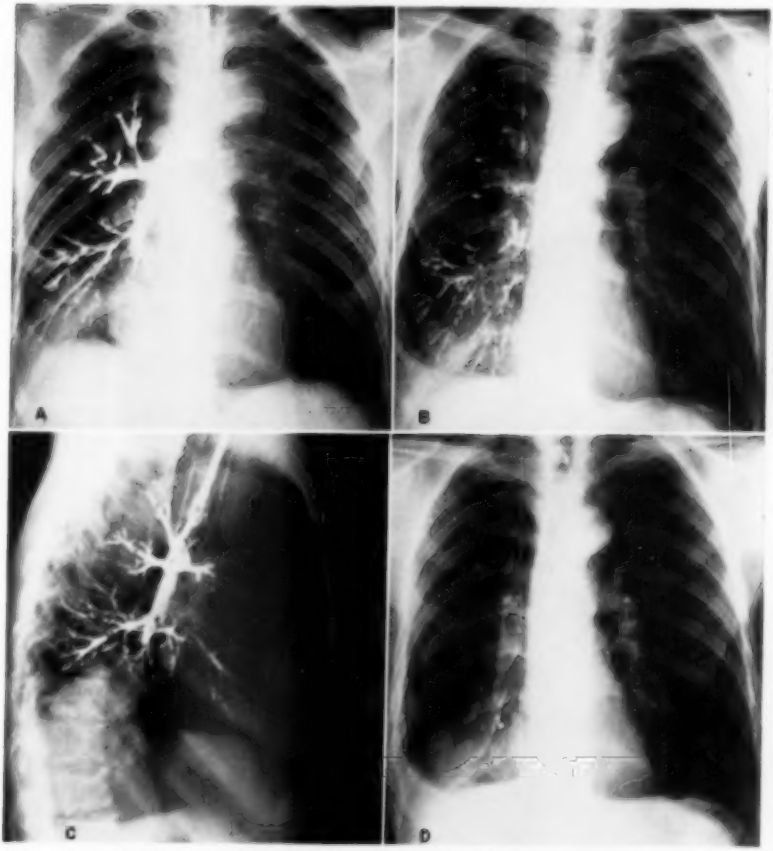

FIG. 2. Bronchograms to investigate density in the right lower lung using 12 ml. of oily Dionosil.

A. Film, obtained with the patient in the recumbent position, shows filling of right lung.

B. Film demonstrates coating of the bronchial tree, with the patient in the upright position. This film was taken following voluntary cough and does not show "alveolar filling."

C. Right lateral film demonstrates occlusion of basal segments of right lower lobe.

D. Film shows amount of clearing one hour after bronchography.

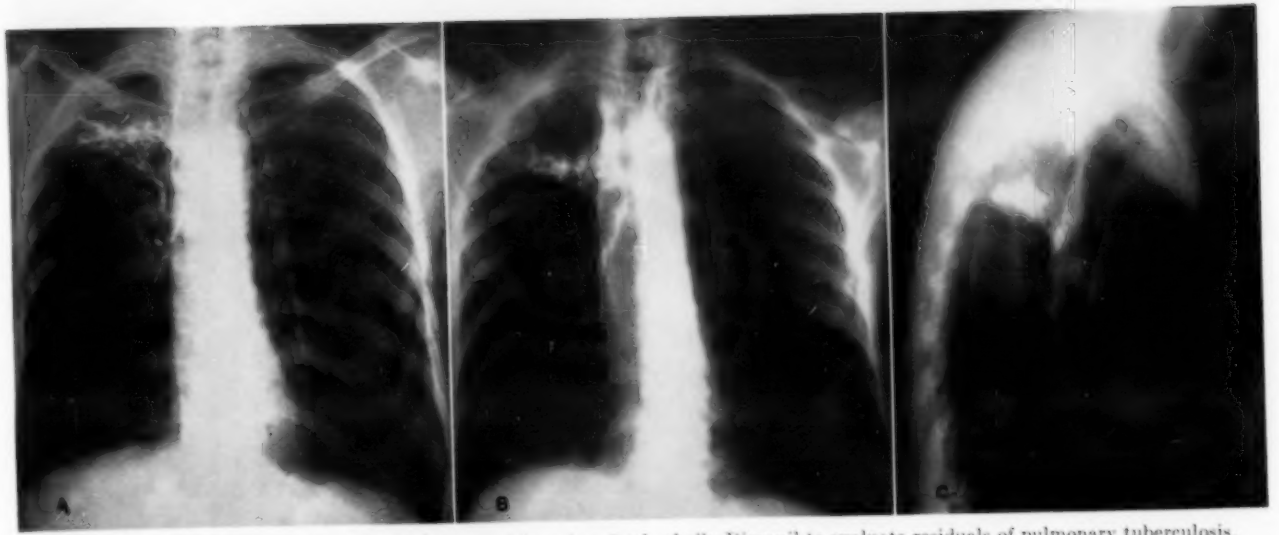

FIG. 3. Selective bronchography of the right upper lobe using 40 ml. of oily Dionosil to evaluate residuals of pulmonary tuberculosis.

A. Film shows bronchiectatic changes as well as presence of bulla.

B. Laminagram taken at 8 cm. demonstrates type of roentgenogram which can be obtained by this procedure.

C. Right lateral laminagram taken at 11 cm. demonstrates the localization of the observed changes.

FIG. 4. Bilateral bronchography using 40 ml. of oily Dionosil to evaluate the residuals of pulmonary tuberculosis.
A. Presence of large bulla in right upper lobe is demonstrated as well as other bullous and bronchiectatic changes.
B. Right lateral bronchogram obtained before left side was filled.
C. Film shows extent of clearing within twenty-four hours.

BRONCHOGRAPHY WITH NEW CONTRAST MEDIA[1, 2]

A Review

SHELDON E. DOMM,[3] DAVID H. WATERMAN, WILLIAM K. ROGERS, AND CHRISTOPHER CUMMINS

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INTRODUCTION

In the more than thirty years since Sicard and Forestier (1) introduced the use of iodized oil in bronchography, the technique has become more or less standardized and generally satisfactory. The chief drawback has been the occasional persistence of the medium in the alveoli. The current thinking regarding the avoidance of this complication may be considered under two headings: the addition of substances to the iodized oil to render it thicker and, hence, less likely to enter alveoli; and the use of new rapidly eliminated opaque media.

The leading exponents of the "thickened" iodized oil school are the South Africans. Salinger and Houghton (2) present a series of more than 7,000 bronchograms and report the use of Lipiodol® in which sulfanilamide has been suspended. They are convinced of the advantages of the thickened suspension over all other known opaque media.

The use of rapidly eliminated opaque materials has been most enthusiastically embraced on the continent of Europe and in Great Britain. The common chemical factor of the various preparations is iodopyracet (the diethanolamine salt of 3,5-diiodo-4-pyridone N-acetic acid), known in this country as Diodrast.® The two main types of preparation in which the iodopyracet type of substances can be administered are water soluble and oily. Both types of preparation are obtainable in the United States under the trade mark Dionosil® (Glaxo). The active agent of the United States preparations is the n-propyl ester of the iodine-containing organic acid, rather than the diethanolamine salt as in Diodrast. Dionosil aqueous is a 50 per cent suspension in a buffered vehicle containing sodium carboxymethyl cellulose. Dionosil Oily® is a 60 per cent suspension of the ester in peanut oil; the iodine content of the final product is 34 per cent, as compared with 40 per cent for iodized oil. After administration, the ester is slowly hydrolyzed and is then absorbed from the lungs and excreted through the kidneys, being completely eliminated in a short period. There is no breakdown of free iodine.

THICKENED IODIZED OIL

The use of thickened iodized oil was introduced by Dormer and associates (3) in 1945, but did not seem practical to the present writers until the E. Fougera

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[2] Presented at the meeting of the Southern Tuberculosis Conference, Louisville, Kentucky, on September 23, 1955.

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Company introduced the ready-made product Visciodol.[3] This preparation contains 0.32 gm. of sulfanilamide per ml. of Lipiodol, and is ready to use after careful stirring. Although the material has been used by the present writers in too small a series of bronchograms to permit valid conclusions, the experience so far has been rather unsatisfactory. On the basis of experience, it has been observed that the temptation to warm the highly viscid mixture must be resisted lest alveolar filling and retention occur, as warned by Gudbjerg (4). Another disadvantage that has been noted is that actually during the procedure the plunger of the syringe may permanently freeze to the barrel. This phenomenon is associated with the very slow rate at which the material must be injected.

The films made with the aid of Visciodol are of good quality, but alveolar filling is not altogether prevented. As Bariety and associates (5) have observed, one may also encounter the false appearance of amputation if the films are taken too soon. They also mention the difficulty of progress in certain bronchi, e.g., those with lacerated walls or of small caliber.

These considerations led Boet (6) to conclude that every diagnostic problem requires a Lipiodol preparation of a different composition. But, for the sake of simplicity, he limits himself to three concentrations, zero, 15 per cent, and 25 per cent sulfadiazine.

Calix (7), on the other hand, states that he routinely gets excellent films using Visciodol. In spite of the fact that he warms the mixture, he has not encountered alveolar filling or retention, and therefore considers the medium much better than the unthickened iodized oil. Calix believes that the use of this preparation requires deep topical anesthesia to the point of abolition of the cough reflex. He uses 5 per cent Cyclaine® for anesthesia.

Rayl (8) has had good experience with Visciodol and prefers it to all other media. He does not abolish cough; rather he sometimes utilizes the cough mechanism to bring about a more peripheral bronchial distribution. Of interest is his observation that objectionable retention is not absolutely prevented by the use of the sulfanilamide-Lipiodol agent. Rayl estimates that possibly 15 per cent of patients will show this complication. He believes, as does Gudbjerg that, if the opaque medium is retained in the alveoli for a month or so, it persists as long as does the regular Lipiodol. Rayl believes that Visciodol in his hands yields better bronchograms than does Dionosil Oily or unthickened iodized oil.

Of some interest, possibly, is the observation by the patients that Visciodol has an unpleasant taste.

DIONOSIL OILY

The use of Dionosil Oily by the present writers has proved more practical than the use of Visciodol. In a series of more than 100 bronchograms, the films have been of acceptable quality, the material has been easy to handle and administer, is acceptable to the patient, and has produced no ill effects. The lungs are clear of opaque material in a day or two. Dionosil Oily shows less tendency toward alveolar filling than does iodized oil, regular or thickened. This characteristic would seem to be due to its tendency to adhere to surfaces rather than

to its viscosity, which is actually low. The fact that Dionosil Oily seems to stick to bronchial and bronchiolar walls also renders it less likely to be cleared by untimely cough. This trait makes it particularly acceptable to the bronchographer, needless to say.

It should be pointed out that, by the same token, Dionosil Oily takes longer to reach the periphery of the bronchial tree and adheres in unexpectedly large quantities to the nose if introduced transnasally, to the throat if put in supraglottically, and to the trachea and/or bronchi by any technique. In other words, more medium is used than is the case with iodized oil. Moreover, at present the cost of Dionosil Oily is considerably higher than that of Iodochlorol*, so that the cost of the medium in practice is probably twice that of iodized oil.

Dionosil Oily is especially suitable for bronchography in children under general anesthesia in view of the higher tendency toward alveolar filling under such circumstances.

DIONOSIL AQUEOUS

The interest of the present writers in using water-soluble contrast media was somewhat dulled by the report of three deaths (9). (In a series of more than 1,500 bronchograms in the writers' practice, using Iodochlorol, there have been no deaths.) The report by Shefts (10) of three cases of pneumonitis following the use of aqueous Dionosil was also disturbing. (Dr. Shefts has subsequently changed to Dionosil Oily.) Furthermore, meticulous fluoroscopic control of the advancing opaque medium has always been advocated by the present writers. This maneuver is not always possible when the very rapidly eliminated aqueous product is used.

* * *

It is the belief of the present writers that there is a trend away from the use of the rapidly eliminated preparation to the use of the oily one. Cummins states that in the Thoracic Surgical Unit at Papworth, England, the use of Dionosil Oily has been routine since the publication of the paper by Cummins and Silver (11) in August, 1953, and that he has observed no granulomatous formation. Forgan-Morle and Robertson (12) report a series of 500 bronchograms with Dionosil Oily by the crico-thyroid method. They have abandoned the use of the aqueous preparation and of iodized oil preparations. In another recent British report, Mackay-Dick likewise advocates Dionosil Oily. He also uses the crico-thyroid route. Conway Don (14), after a series of 60 bronchograms with aqueous Dionosil, concludes that it is not practical. Wishoff and Felson (15) believe that the oily product will become the material of choice in bronchography. Norris and Stauffer (16) also recommend Dionosil Oily, but observe that one should allow twice as much time as in the case of iodized oil. McSwan and Allan (17) enjoyed consistently good results with Dionosil Oily as did Holden and Crone (18). McLaren (19) states that the use of Dionosil Oily is now routine at Duke University after an unsatisfactory experience with aqueous Dionosil.

Flipse (20), on the other hand, believes that Dionosil Oily is no better than the aqueous, which has proved highly satisfactory in his hands in a large series over

a number of years, both prior to the publication of his paper in February, 1953 (21), and subsequently.

In so far as thickened iodized oil is concerned, the advantage over the unthickened iodized oil would appear to be quantitative rather than absolute, as observed by Rayl. That is to say, one must anticipate a small but significant incidence of objectionable retention. Whether this quantitative advantage renders it preferable as compared to regular iodized oil is a matter for each worker to decide. Certainly, in cases in which risk of retention must not be taken, Dionosil rather than Visciodol should be used.

ANESTHESIA

The interest of the present writers in modifying their bronchographic routine was not primarily aroused by the problems of alveolar retention, which has not proved troublesome. Rather, the greater concern has been with the occasional incidence of reactions which, although never fatal or serious, have certainly been a cause of distress. The question whether the anesthetic agent or the iodized oil, or both, was responsible was not always clear although true iodism has not definitely occurred (being, fortunately, apparently very rare) (22, 23). The writers believe that freshly made 10 per cent cocaine has been an ideal agent from the standpoint of safety and good anesthesia. In approximately ten thousand bronchoscopies done by the present writers when cocaine was used, there has not been a single fatality, although there have been occasional reactions. In office bronchography, however, the difficulty of having to make up cocaine solution fresh each time and to keep narcotic records makes the temptation to use a prepared stable solution, such as tetracaine hydrochloride (Pontocaine®), a very real one. For this reason tetracaine hydrochloride has been used as an anesthetic agent in an appreciable number of bronchographies, although the toxicity and danger have been recognized. Six deaths from this drug have recently been reported by other workers in this area, two of these deaths reportedly having been in connection with bronchography. Consequently, extreme care has been used in connection with the dosage. The work of Carabelli (24) has greatly clarified the procedure in this regard. Since adopting his "subposologic" quantities of anesthetic, particularly since using Dionosil Oily, there have literally been no significant reactions. Carabelli advises the use of no more than 8 ml. of 0.25 of tetracaine hydrochloride (20 mg.). With the expenditure of sufficient time, it has usually been possible to obtain satisfactory although slow-acting anesthesia with 8 ml. or less in bilateral mapping. Nevertheless, the introduction of faster acting and safer anesthetic agents is helpful.

At the present time the writers are using 5 per cent hexylcaine hydrochloride which apparently carries a much higher safety factor and offers the advantage of a stable readily available solution not subject to narcotic regulation. It may supplant the use of tetracaine hydrochloride if the safety factors are borne out by long experience.

General anesthesia in bronchography also remains a problem, apparently

demanding a high degree of art on the part of the anesthesiologist. In our opinion the ideal anesthetic agent has not been found.

TECHNIQUE

The writers have always believed that there is no single method for obtaining bronchograms. Many excellent methods have been described over the years and many poor bronchograms have sometimes resulted. It is believed that bronchography is an art and not a science, i.e., a man should find a method that suits him and stick to it.

Dionosil Oily and Visciodol may be administered by any technique preferred by the operator. Their stickiness tends to enhance the value of a tracheal catheter or large-bore transcrico-thyroid needle as compared to the circumstances obtaining with iodized oil. It is important to keep the technique of outpatient office bronchography as simple as possible. For this reason the present writers for twelve years have used the transnasal route for both topical anesthetic and contrast medium, as advocated by Carr (25) in this country and by Forestier and Leroux abroad. After introduction of a small amount of anesthetic agent through a medicine dropper in the nose, with the patient breathing deeply and regularly, the opaque medium is injected through a short soft rubber tube into the nostril and is allowed to run down the pharynx, spilling over into the trachea. If the patient is well positioned to the side to be filled and somewhat tilted backward, it is possible very satisfactorily to fill the lower portion of the bronchial tree on this side. About two minutes in the knee-chest position with the shoulder of the side to be filled against the floor suffices to fill the upper lobe. The filling is then checked with fluoroscopy, and a six-foot erect lateral film taken. Immediately following this postero-anterior stereo films are obtained.

If the patient is not emphysematous and experiences no difficulty from the procedure, the other side is filled in the same manner without repeating the anesthetic agent. Instead of a lateral film an oblique one is taken, as well as another set of postero-anterior stereos.

It has been the experience that satisfactory filling of all five lobes can be achieved in a rather high percentage of cases. It is imperative that each segment of each lobe should be filled, as it is obviously as important to know what segments are normal as to know which are abnormal. Occasionally additional oil will be necessary to delineate a given segment.

Immediately following the completion of the roentgenograms, the patient is given postural drainage and then told for the first time to cough. The patient again takes postural drainage when he returns home, later in the afternoon, and again before going to bed. Usually this suffices to remove the oil. It seems that the transnasal route requires less anesthetic agent than the tracheal catheter method, and produces less cough during the procedure.

In hospital practice Cummins prefers the use of the tracheal catheter, as does the bronchography department at the East Tennessee Tuberculosis Hospital. The writers have had no experience with the crico-thyroid needle method. It might be mentioned that, regardless of technique or medium, a prior bronchos-

copy followed by a week or so of postural drainage facilitates bronchial hygiene and permits study of bronchial abnormalities affecting segmental filling.

Using the transnasal method with postural drainage following the bronchography as outlined, alveolar retention of Iodochlorol has been so rare as to not constitute a real problem.

SUMMARY

The armamentarium of the bronchographer has been increased by the addition of new contrast media to the basic iodized oil preparation. He can now select the medium best suited to the diagnostic problem, the local situation, and his own experience.

Iodized oil thickened with sulfanilamide is proving a valuable agent in experienced hands. It markedly reduces but does not altogether eliminate the complication of objectionable alveolar retention.

The present writers are not aware of an aqueous contrast medium that meets the requirements regarding safety, anesthesiologic simplicity, and fluoroscopic control.

The use of Dionosil Oily® eliminates the chief defects of iodized oil preparations, i.e., occasional alveolar filling and retention. Its use has been found to be safe and satisfactory by any technique. Indications for the use of rapidly eliminated media include cases in children, preoperative cases, pulmonary tuberculosis, cases to be diagnosed roentgenographically, medico-legal cases, emphysema, and iodine sensitivity cases. Disadvantages of Dionosil Oily include its greater cost and slower flow.

Iodized oil, particularly Iodochlorol®, remains a very good agent. With good transnasal technique and subsequent postural drainage, alveolar filling and retention are uncommon. Iodized oil, unthickened, is also useful for mapping segments which are difficult to fill.

Anesthesia remains a problem in bronchography. Cocaine is satisfactory but inconvenient. Tetracaine hydrochloride is toxic in ordinary concentrations but is safer in concentrations of 0.25 or 0.50 per cent, although correspondingly slower acting and of shorter duration. The use of a tracheal tube requires deeper anesthesia than the transnasal route. Hexylcaine hydrochloride is currently under clinical investigation and shows considerable promise. Careful selection of cases, use of "subposological" quantities of topical anesthetic agent, and the use of barbiturates and antihistaminics reduce but do not eliminate the hazard of reaction to anesthetic agent or opaque medium.

SUMARIO

La Broncografía con Nuevos Medios de Contraste

El arsenal del broncógrafo se ha enriquecido con la adición de nuevos medios de contraste a la preparación básica de aceite yodado, pudiendo ahora escoger el medio más ajustado al problema diagnóstico, a la situación local y a su propia experiencia.

El aceite yodado espesado con sulfanilamida va resultando ser un agente valioso en manos avezadas. Aunque reduce notablemente, no elimina del todo la complicación de una retención alveolar contraproducente.

Los AA. no tienen conocimiento de ningun medio acuoso de contraste que cumpla los requisitos relativos a inocuidad, sencillez anestesiológica y gobierno roentgenoscópico.

El empleo del Dionosil Oleoso® elimina los principales defectos de las preparaciones de aceite yodado, o sean, henchimiento y retención ocasionales en los alvéolos. Su uso ha resultado inocuo y satisfactorio con cualquier técnica. Las indicaciones para el empleo de medios de rápida eliminación comprenden casos en niños, casos preoperatorios, tuberculosis pulmonar, casos para diagnóstico roentgenográfico, casos médico-forenses, enfisema y sensibilidad al yodo. Las desventajas del Dionosil oleoso® comprenden el mayor costo y paso más lento.

El aceite yodado, y en particular el Yodoclorol®, continúa siendo un agente muy bueno. Con una buena técnica transnasal y drenaje postural subsiguiente, el henchimiento y la retención alveolares son raros. El aceite yodado, sin espesar, es además útil para delinear segmentos que es difícil llenar.

La anestesia continua siendo un problema en la broncografía. La cocaína es satisfactoria, pero inconveniente. El clorhidrato de tetracaína es tóxico a las concentraciones ordinarias, pero inocuo a concentraciones de 0.25 ó 0.50 por ciento, aunque correspondientemente de acción más lenta y más breve. El empleo de una sonda traqueal requiere anestesia más profunda que la vía transnasal. El clorhidrato de hexilcaína se halla actualmente bajo investigación clínica y ofrece mucha promesa. La cuidadosa selección de casos, el uso de cantidades "subposológicas" de un anestésico local y el uso de barbitúricos y antihistamínicos reducen, pero no eliminan, el riesgo de reacciones al anestésico o al medio opaco.

RESUME

Bronchographie avec un nouveau milieu de contraste

L'arsenal de la bronchographie s'est enrichi d'un nouveau milieu de contraste qui s'ajoute à la préparation d'huile iodée fondamentale. Le spécialiste peut maintenant choisir le milieu le mieux adapté au problème diagnostique, aux conditions locales et à sa propre expérience.

L'huile iodée épaissie par le sulfanilamide est un agent précieux entre des mains expérimentées. Elle réduit nettement, sans toutefois l'éliminer completement le danger que représente la rétention alvéolaire.

Ces auteurs n'ont pas connaissance d'un milieu aqueux opaque aux rayons X qui satisfasse aux exigences du point de vue sécurité d'emploi, simplicité anesthésiologique et valeur radioscopique.

L'emploi du Dionosil huileux® élimine les inconvénients principaux des préparations huileuses, c'est à dire, occasionnellement, le remplissage alvéolaire et la rétention. Quelle que soit la technique, son emploi s'est montré satifaisant et sans danger. Les indications des substances rapidement absorbées comportent l'utilisation chez l'enfant, les cas préopératoires, la tuberculose pulmonaire, les cas réclamant un diagnostic radiographique, les cas de médecine légale, l'emphysème et l'allergie à l'iode. Les désavantages du Dionosil huileux comprennent son prix plus élevé et son écoulement plus lent.

L'huile iodée, en particulier l'Iodochlorol®, demeure un très bon agent. Une bonne technique transnasale et le drainage ultérieur par posture adéquate préviennent habituellement le remplissage de l'alvéole et la rétention. L'huile iodée, non épaissie, sert également à délimiter les territoires difficiles à remplir.

L'anesthésie demeure un problème en bronchographie. La cocaine est satisfaisante, mais incommode. Le chlorhydrate de tétracaine est toxique aux concentrations ordinaires; il offre moins de risques aux concentrations de 0, 25 ou 0, 50%, mais proportionnellement son action est plus longue à se manifester et moins durable. L'application du tube trachéal réclame une anesthésie plus accentuée que la voie transnasale. Le chlorhydrate d'hexylcaine est en cours d'investigation en clinique et ses perspectives sont très satisfaisantes. Une sélection minutieuse des cas, l'emploi de quantités "subposologiques" des agents d'anesthésie locale et l'administration de barbituriques et d'antihistaminiques réduisent, sans les éliminer, les risques de réactions à l'agent anesthésique ou au milieu opaque.

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A PILOT STUDY OF CYCLOSERINE TOXICITY[1]

A United States Public Health Service Cooperative Clinical Investigation

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INTRODUCTION

The usefulness of an antimicrobial agent depends primarily upon its therapeutic effectiveness and its toxicity. A toxic drug with little or no therapeutic effect will obviously be discarded. Conversely an effective drug with few adverse reactions will be assured of acceptance. Many drugs, however, occupying a middle range, are both toxic and efficacious.

The compound D-4-amino-3-isoxazolidone, with the generic name of cycloserine (1), appeared in the early reports to fall in this middle range in which serious toxicity must be weighed against efficacy. Even the first report (2) of the therapeutic efficacy of cycloserine in tuberculous patients was not without some suggestion of neurotoxicity. In other studies, in nontuberculous patients (3), it was noted that the toxic effects were confined to the central nervous system. Since then, additional observations of toxicity in both man (4) and animals (5) have been reported. When the opportunity to evaluate this drug[2] was presented to the clinical investigators participating in the Public Health Service Cooperative Studies, it was agreed that the first step should be a small, but carefully controlled, evaluation of drug toxicity. Investigators in six[3] of the twenty-five cooperating hospitals undertook the pilot study.

MATERIAL AND METHODS

The patients selected for the study had advanced pulmonary tuberculosis and had failed to show satisfactory improvement on other chemotherapeutic regimens. The dearth of treatment available for these patients is apparent from the fact that the investigators were willing to use a drug already reported to have serious toxicity. Patients with a history of mental illness, convulsions, or head injuries were excluded.

Information on the characteristics of the patient, the history of his disease, and his response to previous treatment was submitted to the central office before the beginning of the study for each patient selected by the participating hospitals. Using this information, the central office separated the patients from each hospital into three matched groups. By a system of chance allocation, each group was assigned a different dosage schedule to be

[1] Presented by Dr. Francis J. Murray at the Fifteenth Veterans Administration-Army-Navy Chemotherapy Conference, St. Louis, Missouri, February 8, 1956.

[2] The cycloserine for this study was generously supplied by Merck & Co., Inc., as Oxamycin.

[3] Drs. F. H. Alley, E. Bowerman, and S. C. Carter, Tennessee State Tuberculosis Sanatoriums at Memphis and Oakville, Tenn.; Dr. P. T. Chapman, Herman Kiefer Hospital, Detroit, Mich.; Dr. S. S. Cohen, Glen Lake Sanatorium, Oak Terrace, Minn.; Dr. W. J. Steininger, Wm. H. Maybury Sanatorium, Northville, Mich.; and Dr. D. W. Zahn, Firland Sanatorium, Seattle, Wash.

received for twelve weeks. One-fifth of the patients in each dosage group was then chosen by lot to receive a placebo rather than cycloserine.

One-fourth gram capsules of cycloserine and placebo, identical in appearance, were prepared and packaged in identical bottles by the manufacturer. After each bottle was labeled in the central office with only a patient's name and dosage schedule, the labeled bottles were mailed to the hospitals. Although the staff at each hospital necessarily knew the dosage for each patient, only persons in the central office knew which patients or even how many patients were receiving the placebo. However, each investigator knew that placebos were being used and that information on the contents of the individual bottles must be withheld from him.

At the beginning of the study an additional bottle of placebo capsules was sent to each hospital. It was agreed that, if a patient developed severe signs or symptoms of toxicity, the investigator would discontinue the assigned drug, without knowing whether it was cycloserine or placebo, and, unknown to the patient, would substitute the same dose of placebo capsules for a period of several days. The disappearance of the symptoms would be interpreted as evidence that the symptoms were due to the original medication. The type and severity of the reaction would determine whether the assigned drug would be restarted, reduced in dose, or permanently discontinued.

A total of 143 patients was admitted to the study. Two placebo patients, after an absence from the hospital of three weeks, resumed their assigned medication. Although they continued to take the placebo for twelve weeks, they have been excluded from this analysis. The remaining 141 patients were divided among the treatment schedules as shown in table 1. Of these, 26 patients received various doses of placebo but have been combined in this report into a single group.

In the first four hospitals to enter the study, 1.0 gm. of medication per day was given in two equal doses of 0.5 gm. to 31 patients; 6 received a placebo; and 25, cycloserine. An average dose of 0.5 gm. of medication per day was given to 94 patients in all six hospitals; 17 received placebo and 77, cycloserine. Eight of these patients were given 1.0 gm. of placebo in a single dose every second day and 39 received the same dose of cycloserine. Nine received a single dose of 0.5 gm. of placebo each day and 38 were given an equal amount of cycloserine. By the time the fifth and sixth hospitals entered the study, 1.0 gm. of cycloserine each day already appeared highly toxic. Consequently, 16 patients from these two

TABLE 1

DISTRIBUTION OF 141 PATIENTS BY DOSE AND FREQUENCY OF ADMINISTRATION

Regimen	Number of Patients	
	Placebo	Cycloserine
Total	26	115
One gram per day	6	25
0.5 gm. twice a day	6	25
One-half gram per day	20	90
1.0 gm. every second day	8	39
0.5 gm. once a day	9	38
0.25 gm. twice a day	3	13

hospitals originally scheduled to receive 1.0 gm. a day were given instead 0.5 gm. per day in two doses of 0.25 gm. Three received a placebo and 13, cycloserine.

The five groups of patients were similar with respect to age, race, sex, body weight, and severity of disease. Approximately 95 per cent had far advanced cavitary disease and nearly 90 per cent had had tuberculosis for more than one year. All of the patients had been treated with at least two antituberculous drugs, nearly 80 per cent had received streptomycin, isoniazid, and PAS (in various combinations), and almost 30 per cent had been given pyrazinamide in addition to these three drugs.

Before and after twelve weeks of treatment, each patient had a neurologic examination and a lumbar puncture. Patients in three hospitals also had electro-encephalograms before and after treatment. As earlier investigations reported neurotoxic effects of cycloserine, the patients were examined daily for mental and emotional changes as well as for changes in sensation and neuromuscular control. If signs or symptoms of toxicity appeared, the examinations made at the beginning of the study were repeated. In addition, the clinical and roentgenographic course of the tuberculous disease was observed.

RESULTS

Over-all incidence of drug toxicity: During the twelve-week treatment period, medication for 18 patients was discontinued or reduced to one-half or less of the assigned dose because of signs and symptoms of toxicity. Although the decision to change treatment was made without knowledge of what these patients were receiving, all 18 were in fact receiving cycloserine. The incidence of toxic reactions in the three dosage groups considered severe enough to require a change in regimen is shown in table 2.

Because of severe toxic reactions in several other patients, the dose for 4 patients assigned 0.5 gm. of cycloserine twice a day and 3 patients assigned 1.0 gm. every second day was halved after sixteen days of therapy, even though none of these 7 patients themselves had any symptoms of toxicity. In the analysis, these patients have been included in their original treatment groups with the assumption that, had they continued this dose, no toxicity would have developed. The effect of this assumption is possibly to understate the incidence of toxicity in these two regimens.

Eleven (44.0 per cent) of the 25 patients who received a daily dose of 1.0 gm. of cycloserine had toxic reactions (more than three times the incidence of toxicity observed among all patients who received 0.5 gm. of cycloserine a day). Toxic reactions developed in only 5 (12.8 per cent) of the 39 patients who received 1.0 gm. every second day, in 2 (5.3 per cent) of the 38 patients who received 0.5 gm. once a day, and in none of those who received 0.25 gm. twice a day.

As these 90 patients all received the same average daily dose, it would appear that with the same total dose, the smaller the individual dose, the less frequent were the toxic reactions. None of the patients taking the placebo had signs or symptoms severe enough to require a change in medication.

Type of toxicity: In table 2, the 18 cases of toxicity are classified according to

TABLE 2

TOXIC REACTIONS OF PATIENTS UNABLE TO TOLERATE ASSIGNED CYCLOSERINE REGIMENS

Toxic Reaction	Total	Cycloserine		
		0.5 Gm. Twice a Day	1.0 Gm. Every Second Day	0.5 Gm. Once a Day
Total	18	11	5	2
Convulsion	8	4	3	1
Mental disorder	2	2	—	—
Motor disorder	3	2	—	1
Somnolence	1	1	—	—
Dizziness	3	1	2	—
Fever, chills	1	1	—	—

type of reaction. Eight patients had convulsions with loss of consciousness. Following the seizure each patient received the placebo for at least seven days and during this period had no further symptoms. When 4 of these patients were restarted on one-half the original dose of cycloserine, one had another convulsion, one complained of severe dizziness and diplopia, and 2 had mild symptoms of dizziness which persisted until the drug was reduced to 0.25 gm. daily. The occurrence of convulsions on each regimen suggests that, while a reduction in dose results in a decrease in the frequency of reactions, it does not necessarily lessen their severity.

Of the other 10 cases of toxicity, 8 patients received the placebo in accordance with the protocol and had no symptoms while on placebo. In the other 2 patients, symptoms disappeared when cycloserine was stopped. As each investigator was free to decide whether a patient should resume his assigned medication, the further management of these patients was handled in different ways. Four of the patients received no more cycloserine, 4 resumed treatment with cycloserine at one-half the assigned dose and were then able to tolerate the drug. Symptoms recurred in the 2 patients for whom the original dose of cycloserine was reinstituted.

The indications for change of medication are at least partly subjective, depending on the type and severity of the toxic reactions and the physician's estimate of the risk to the patient. While there can be agreement on an immediate change when a convulsion occurs, it is more difficult to agree on how long or in what degree somnolence, for example, may be allowed to persist before treatment should be changed. Time is the only precisely measurable element in this situation, and both the time when medication was changed and the time when symptoms first appeared were recorded.

Time of appearance of drug toxicity: In table 3 is shown the number of days during which the 18 toxic patients were treated before the assigned cycloserine regimen was discontinued. This period ranges from two to thirty-four days. Although the dose of cycloserine seemed to influence the frequency of toxic reactions, it apparently did not affect the length of time the drug can be tolerated.

TABLE 3

TIME WHEN TREATMENT WAS CHANGED FOR PATIENTS WITH TOXIC REACTIONS TO CYCLOSERINE

Regimen	Total	Days of Treatment				
		1-7	8-14	15-21	22-28	29-35
Total	18	6	7	2	2	1
0.5 gm. twice a day	11	3	6	1	1	—
1.0 gm. every second day	5	2	1	—	1	1
0.5 gm. once a day	2	1	—	1	—	—

The time medication was stopped ranged from the second to the twenty-third day among patients who received 1.0 gm. of cycloserine a day; from the sixth to the thirty-fourth day among patients who received 1.0 gm. every second day; and from the third to the seventeenth day among those who received 0.5 gm. once a day.

Although all 18 patients were removed from the assigned treatment over a span of thirty-four days, symptoms had actually appeared in all of these patients within the first sixteen days. This suggests that most patients who will have a toxic reaction to cycloserine will show some symptoms during the first three weeks of treatment. Instances have been reported in other series (4) in which convulsions occurred after a very much longer course of apparently uneventful administration.

Neurologic findings: The neurologic examinations before and after treatment were made without knowledge of whether a patient was receiving cycloserine or the placebo. A summary of the results of these examinations is presented in table 4. For each sign and symptom, the number of patients is shown for whom an initially normal response was abnormal at the end of the twelve-week course of treatment; or, if initially abnormal, was subsequently exaggerated. The same patient may appear more than once in this table and, indeed, 3 patients had eight abnormal responses.

Abnormal responses were slightly more frequent among the patients who had to stop taking cycloserine, but there was no concentration of abnormalities of a particular type. No pattern of abnormalities or greater frequency among the cycloserine patients was discernible to the investigators, but the material is made a matter of record for comparison with other series.

Cerebrospinal fluid findings: The study plan included at least two cerebrospinal fluid examinations for every patient, one examination before and one following the twelve-week course of treatment. This requirement was not met for 16 of the 141 patients because of the patients' refusal to submit to a second lumbar puncture or because no fluid could be withdrawn. Each specimen of cerebrospinal fluid was analyzed in the hospital laboratory without knowledge of the patient's treatment regimen or whether he had had a toxic reaction.

The cerebrospinal fluid protein determinations for 125 patients are presented

TABLE 4

NUMBER OF PATIENTS, BY REGIMENS, IN WHOM ABNORMAL RESPONSES ON NEUROLOGIC EXAMINATION APPEARED OR INCREASED DURING A TWELVE-WEEK COURSE OF TREATMENT

Item	Placebo	Cycloserine						
		0.5 Gm. Twice a Day		1.0 Gm. Every Second Day		0.5 Gm. Once a Day		0.25 Gm. Twice a Day
		Non-toxic	Toxic*	Non-toxic	Toxic*	Non-toxic	Toxic*	
Number of patients studied	25	14	11	33	5	35	2	12
Alertness							1	
Cooperativeness						1		
Insight			1	1		1	1	1
Apprehension	2	1	3	1	1	1	2	
Phobia	1							
Illusions	1			1				
Delusions	1	1		1				
Depression		1		1		1		
Somnolence				1			1	
Orientation		1	1					
Memory defect	4		4	8	1	3	2	
Speech defect	4		5	7	1	2	1	
Smell							1	
Gross fields			1					
Fundoscopic	1	1	2	3		3		
Corneal reflex				1				
Taste		1		2	2	2		
Vertigo	1		1				1	
Hearing acuity			1	1		2		1
Weber	2	2		4		3		
Rinne	2				1	2	1	1
Gag reflex		1		2	1	1		
Uvula deviation	1		1	1				
Trapezius, unequal						1		
Tongue deviation	1	1		1		1		
Tongue tremor		1		1		2		1
Tone—muscle						1		
Atrophy—muscle	1		1			1		
Tremor—muscle	1	1						
Finger to nose	1			1		1		
Heel to knee	1		1	2				
Past pointing	1	1		2				
Gait			1				1	
Grip strength	1					1		
Biceps strength	1			1		1	1	
Quadri strength	1		2	1				
Ankle strength	1		2	2				
Reflex, biceps	1	1	1		1	1		
Reflex, triceps	1	2	1	1	1	4		
Reflex, patella	1	1	1	2	1	2		2
Reflex, ankle	3	1	2	3	2	2		1
Reflex, abdominal	3			4		2		1
Reflex, cremasteric	2			2	1			
Reflex, Babinski				1				
Reflex, Hoffman	1			1				1
Reflex, Chvostek						1		
Sensation		1		1		3		

* Patients for whom cycloserine was discontinued or reduced in dosage

TABLE 5

DISTRIBUTION OF 125 PATIENTS BEFORE AND AFTER TWELVE WEEKS OF TREATMENT BY AMOUNT OF CEREBROSPINAL FLUID PROTEIN

	Milligrams of Protein per 100 Ml.							
	0	10	20	30	40	50	60	70
Patients treated with placebo								
Pre-study 22	—	3	2	10	6	1	—	—
Post-study 22	1	2	7	5	5	2	—	—
Patients with no toxic reaction to cycloserine								
Pre-study 89	—	10	25	30	20	3	—	1
Post-study 89	1	15	25	26	18	3	1	—
Patients with toxic reactions to cycloserine								
Pre-study 14	—	—	6	5	3	—	—	—
Post-study 14	—	—	5	4	4	—	1	—

in table 5. The patients have been divided into three groups: 22 patients who received the placebo, 89 who received various doses of cycloserine but had no evidence of toxicity, and 14 who had toxic reactions to cycloserine. The amount of protein in the cerebrospinal fluid ranged from less than 10 mg. per 100 ml. to slightly more than 70 mg. per 100 ml. The distribution of patients within this range was the same in all three groups both before and after treatment. There was no suggestion that cycloserine altered the amount of protein in the cerebrospinal fluid of the patients who took the drug for twelve weeks. This finding is in agreement with the report of Epstein and associates (6).

In addition to the pre-study and post-study cerebrospinal fluid examinations, 14 of the 18 patients who had toxic reactions had lumbar punctures at the time of their reactions and there was no indication that the amount of protein was increased at that time.

Electro-encephalograms: Three of the hospitals had access to facilities for making electro-encephalograms, and tracings were made before and after treatment for each of the 64 study patients in these hospitals. The central office submitted the series of tracings to a consulting electro-encephalographer, Dr. Curtis Marshall of The Johns Hopkins University.

Dr. Marshall first evaluated each tracing independently and in a random order, knowing the sex and pentad of age of each patient but without knowledge of the patient's regimen or whether the tracing was made before or after treatment. For the analysis, his interpretation of the post-treatment tracing was compared with his interpretation of the pretreatment tracing for the patient.

Each pair of interpretations was classified as showing *improvement*, *no change*, or *worsening*. A classification of *improvement* indicates that the post-treatment tracing was interpreted as less abnormal than the pretreatment tracing; *no change*, that the two interpretations were the same; and *worsening*, that the post-treatment tracing was interpreted as more abnormal than the pretreatment

TABLE 6

INTERPRETATIONS OF ELECTRO-ENCEPHALOGRAMS MADE BEFORE AND AFTER TREATMENT ON 64 PATIENTS

Change from Pretreatment to Post-Treatment	Each Tracing Evaluated Independently			Comparison of Post-Treatment Tracing with Pretreatment			Comparison Knowing Toxicity and Treatment
	Placebo	Cycloserine		Placebo	Cycloserine		
		Nontoxic	Toxic		Nontoxic	Toxic	Cycloserine Toxic
Improvement	—	7	4	—	2	—	—
No change	5	27	5	7	22	8	4
Worsening	6	8	2	4	18	3	7

tracing. A summary of this comparison is presented in the first three columns of table 6 for 11 patients who received the placebo, 42 who received cycloserine in various doses, and 11 who had a toxic reaction to cycloserine.

When compared in this manner, the tracings of patients who received the placebo showed worsening after treatment as frequently as those who received cycloserine. Considering the small number of patients in each group, the distribution of toxic patients is not different from nontoxic patients who received cycloserine. Although various doses of cycloserine have been combined in this table, the results on each regimen were examined separately, and there was no suggestion that a high dose of cycloserine resulted in more changes in the tracings than a low dose.

Dr. Marshall then read the tracings a second time, knowing, in addition to the sex and pentad of age for each patient, whether a tracing was made before or after treatment. Without reference to the results of his previous reading, he compared the pretreatment and post-treatment tracings directly and classified the latter as showing improvement, no change, or worsening. The results of this comparative reading are presented in the second three columns of table 6. With this method of reading, there are again no significant differences among the three treatment groups. The second tracing of nearly two-fifths of the patients was more abnormal than the first, and most of the others showed no change.

The results of the two methods of evaluation may be compared. When each tracing was read independently, the post-treatment tracings for nearly 20 per cent of the patients showed some improvement over the pretreatment tracing and only 25 per cent showed worsening. However, when the reader directly compared the post-treatment tracing with the pretreatment tracing, only 3 per cent had improved and nearly 40 per cent were interpreted as showing worsening.

The comparison may be carried one step further. Tracings from the 11 toxic cases were read a third time with the added information of regimen and type of toxic reaction. The results of this reading are shown in the third part of the table. In only 4 patients was the electro-encephalogram interpreted as showing no change and, in 7, the tracings were interpreted as showing an unfavorable change.

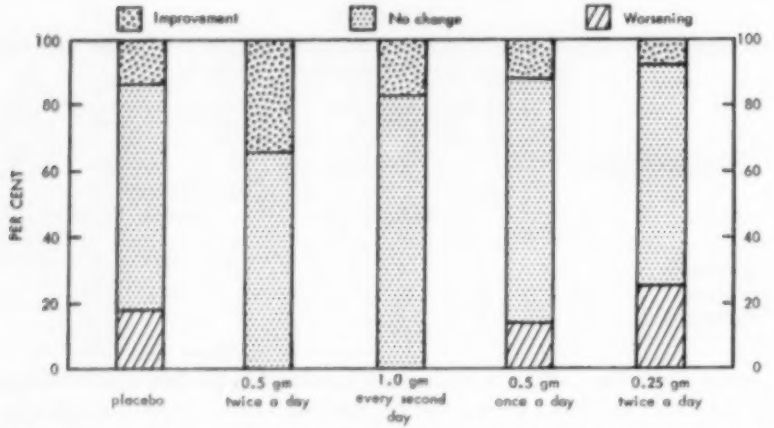

FIG. 1. Percentage distribution of patients by roentgenographic changes observed after twelve weeks on each regimen.

Roentgenographic and bacteriologic findings: Although this study was primarily designed to evaluate the toxic effects of cycloserine, roentgenograms were taken and specimens of sputum were cultured every four weeks during the course of treatment. Patients for whom the prescribed cycloserine regimen was discontinued, either because they showed toxic reactions or because the investigators believed the regimen was demonstrably toxic, have been excluded from the summary of roentgenographic and bacteriologic change.

Roentgenograms for each patient were read serially for change by a single reader in the central office, with no knowledge of the hospital, patient, drug, or dose being received. The changes observable by roentgenogram at the end of twelve weeks of treatment among patients who continued on their assigned regimen for the entire period are shown in figure 1. These roentgenographic results should be interpreted with caution because the number of patients in each group is small and nearly all of these patients had only an extremely limited potential for improvement.

The first bar in figure 1 shows the roentgenographic changes observed in the placebo patients after twelve weeks of observation. Approximately 10 per cent had improved and approximately 20 per cent had grown worse, while the remainder had shown no change. This may be regarded as a "yardstick" of sorts against which the effect of various cycloserine regimens may be measured. The most toxic regimen, 0.5 gm. twice a day, was the most effective therapeutic regimen for the few who continued this regimen for the entire twelve weeks. Three of the 9 patients showed slight improvement of their disease and in none did it grow worse. The second most toxic regimen, 1.0 gm. every other day, was also the second most therapeutically effective regimen; 6 or approximately 25 per cent of 25 patients improved and none grew worse. On the two other regimens, 0.5 gm. daily and 0.25 gm. twice a day, the proportion of patients who

improved or whose disease grew worse was essentially the same as in the group of patients receiving the placebo.

With the exception of one case in the placebo group, the degree of roentgenographic improvement noted was only slight; and the worsening was, in the main, only slight. It is unfortunate that the only regimens on which the therapeutic results observable by roentgenogram were superior to those obtained with the placebo were the toxic regimens. Their value, it appears, was as much in keeping these patients from getting worse as in producing improvement.

Bacteriologic material is scanty, being further limited to patients who were initially producing tubercle bacilli. However, reversal of infectiousness may be, particularly in the type of case studied here, a better measure of a drug's effectiveness than roentgenographic change. Many of these patients had already experienced such extensive destruction of lung tissue that the possibilities for improvement observable by roentgenogram were extremely limited; on the other hand, every patient producing tubercle bacilli in his sputum was a candidate for bacteriologic improvement. Therefore, some note should be taken of the fact that treatment with cycloserine resulted in reversal of infectiousness twice as often as treatment with the placebo: 30 (45 per cent) of 66 cycloserine patients and 4 (21 per cent) of 19 placebo patients. Although the numbers are obviously small, there was no apparent trend for the larger doses to be more effective.

DISCUSSION

This study has accomplished its primary purpose, to obtain information on the frequency and severity of toxic reactions to cycloserine. The hope, although not the expectancy in view of earlier reports, had been to find this new antimicrobial agent therapeutically effective and essentially nontoxic. Failing this, it would have been desirable to find that, by restriction to certain kinds of patients, low doses, or short-term therapy, toxicity could be avoided but therapeutic efficacy retained.

For example, if toxicity occurred only among patients with certain identifiable characteristics, pretreatment screening could select patients who might safely be treated with cycloserine. Unfortunately, careful scrutiny of the material has failed to disclose any association between the occurrence of toxicity and the patients' age, race, sex, or neurologic responses.

It would have been encouraging to have found that the occurrence of toxicity was confined to a certain dosage level and that lower doses were still in the effective range. It is true that decreasing the size of the individual dose appeared to reduce the incidence of toxic reactions. However, the dose which was nontoxic was not demonstrably therapeutically effective, at least by roentgenographic criteria. Moreover, decreasing the dose did not reduce the severity of the toxic reactions; on even the least toxic of the three toxic regimens, one of the 2 patients unable to tolerate cycloserine had a convulsion.

If residual amounts of cycloserine gradually accumulate in the body and result in toxic reactions only when a critical level is reached, the drug might safely be used for a short period of time which would be long enough to obtain a thera-

peutic effect. Unfortunately, this does not appear to be the mechanism of cycloserine toxicity, as toxic reactions occurred during the first week on all three toxic regimens.

Inclusion of a placebo in the trial proved useful in several ways. Thus, it was possible to evaluate the large subjective element in neurotoxic reactions and to protect both the subject and the observer from unconscious bias. Even though neither patient nor physician knew if a patient were receiving cycloserine or the placebo, no patient on placebo developed toxic reactions severe enough to require a change from the assigned regimen. This strengthens the evidence of the reality and severity of the eighteen toxic reactions among patients receiving cycloserine.

Another advantage of using the placebo was that it allowed the investigator to substitute a placebo when an undesirable reaction occurred. The disappearance of symptoms when the patient did not know the placebo had been substituted and when the physician did not know whether he had actually discontinued cycloserine or placebo further corroborates the reality of the toxicity.

Without the placebo, erroneous conclusions could have been reached on the results of the neurologic and cerebrospinal fluid examinations. Finally, inclusion of the placebo regimen made it possible to discern that, although cycloserine did not produce dramatic roentgenographic improvement in "treatment failures," it did apparently prevent deterioration.

The fact should not be overlooked that no toxicity was observed among the 13 patients to whom cycloserine was given in doses of 0.25 gm. twice a day. While this dose was apparently no more effective than the placebo as judged by roentgenographic changes, it might be an adequate dose in combination with another antimicrobial agent. On the other hand, its safety certainly cannot be assumed without further trial on a larger number of patients.

SUMMARY

A control study on the toxic effects of cycloserine was conducted in six hospitals on 141 tuberculous patients in such a way that neither patients nor physicians knew whether a particular patient was receiving drug or a placebo. Most of the patients had far advanced and cavitary pulmonary tuberculosis and were in very poor condition despite extensive previous chemotherapy.

The cycloserine regimens investigated were 0.5 gm. twice a day, 1.0 gm. in a single dose every second day, 0.5 gm. daily in a single dose, and 0.25 gm. twice a day. Neurologic and cerebrospinal fluid examinations were made before and after a twelve-week course of treatment. In three hospitals, electro-encephalograms were also made at the beginning and end of the observation period. The patients were examined daily for psychic and neurologic changes. Reports on clinical, bacteriologic, and roentgenographic status were made monthly.

During the twelve-week course, toxic reactions severe enough to necessitate departure from the assigned regimen occurred in 18 patients who received cycloserine and in none of the placebo patients. Eight patients had convulsions; 2, mental disorders; 3, motor disorders; one, somnolence; 3, dizziness; and one, fever and chills. Toxic reactions occurred on three of the four cycloserine regi-

mens: in 11 of the 25 patients who received 0.5 gm. twice a day, in 5 of the 39 who received 1.0 gm. every second day, and in 2 of the 38 patients who received 0.5 gm. once a day.

Although the assigned cycloserine regimen had to be discontinued for 18 patients during the first thirty-four days of treatment, none of those who passed the thirty-fourth day without incident developed toxic reactions during the remainder of the twelve-week treatment period.

No systematic increase in cerebrospinal fluid protein was noted in relation to either treatment with cycloserine or toxic reactions to the drug. Electro-encephalograms did not prove useful in identifying before treatment the patients who subsequently developed toxic reactions, nor did they reveal residual damage after the occurrence of the toxic reactions. The neurologic examinations did not reveal any consistent pattern of abnormalities.

While the roentgenograms of the cycloserine patients did not reveal striking improvement during treatment, comparison with the roentgenograms of patients who received the placebo indicated that cycloserine apparently prevented further deterioration. However, this limited therapeutic effect was observed only on regimens which produced toxic reactions. Bacteriologically, cycloserine appeared more definitely effective; infectiousness was reversed in 30 (45 per cent) of 66 cycloserine patients and in only 4 (21 per cent) of 19 placebo patients.

Cycloserine produced serious toxic reactions in 18 of 115 patients. It possessed a measure of therapeutic effectiveness as indicated by absence of roentgenographic deterioration and by a moderately high bacteriologic conversion rate. The excellence of other available antimicrobial combinations and the toxicity of cycloserine will probably preclude its use for initial treatment. However, its usefulness in small doses, either alone or in combined therapy, for patients who have failed to respond adequately to combinations of isoniazid, streptomycin, and PAS must be determined by further trials.

SUMARIO

Estudio Explorador de la Toxicidad de la Cicloserina

En seis hospitales se llevó a cabo en 141 tuberculosos un estudio comprobador de los efectos tóxicos de la cicloserina de tal modo que ni los enfermos ni los médicos supieran si un sujeto dado recibía la droga o un placebo. La mayor parte de los enfermos tenía tuberculosis pulmonar muy avanzada y cavitaria y se hallaban en estado muy malo a pesar de una intensa quimioterapia anterior.

Los regímenes de cicloserina investigados fueron de 0.5 gm. dos veces diarias, 1.0 gm. en una sola dosis cada dos días, 0.5 gm. diarios en una sola dosis y 0.25 gm. dos veces diarias. Antes y después de una serie de doce semanas de tratamiento se hicieron exámenes neurológicos y del líquido céfalorraquídeo. En tres hospitales, se tomarón además electro-encefalogramas al principio y al fin del período de observación. Los enfermos fueron examinados a diario en cuanto a alteraciones psíquicas y neurológicas. Cada mes se prepararon informes relativos al estado clínico, bacteriológico y roentgenográfico.

Durante la serie de doce semanas, hubo reacciones tóxicas de suficiente gravedad para imponer desviaciones del régimen establecido en 18 enfermos que recibían cicloserina, sin observarse esto en ninguno de los que recibían placebos. Ocho enfermos tuvieron convulsiones; 2, trastornos mentales; 3, trastornos motores; uno, somnolencia; 3, vértigo; y uno, fiebre y calofríos. Hubo reacciones tóxicas con tres de los cuatro regímenes de cicloserina:

en 11 de los 25 que recibían 0.5 gm. dos veces diarias, en 5 de los 39 que recibían 1.0 gm. cada dos días y en 2 de los 38 que recibían 0.5 gm. una vez al día.

Aunque el régimen de cicloserina fijado tuvo que abandonarse en 18 enfermos durante los primeros treinta y cuatro días de tratamiento, ninguno de los que pasaron el trigésimocuarto día sin contratiempo manifestó reacciones tóxicas durante el resto del período de doce semanas de tratamiento.

No se notó aumento sistemático de la proteína del líquido céfalorraquídeo en relación ya con el tratamiento con cicloserina o con las reacciones tóxicas a la droga. Los electroencefalogramas no resultarón útiles para identificar antes del tratamiento a los enfermos que manifestaron después reacciones tóxicas, ni tampoco revelaron lesiones residuales después de aparecer reacciones tóxicas. Los exámenes neurológicos no revelaron ningún patrón constante de anomalías.

Aunque las radiografías de los enfermos tratados con cicloserina no mostraron notable mejoría durante el tratamiento, una comparación con las de los que recibieron el placebo indicó que la cicloserina impedía aparentemente mayor deterioro. Sin embargo, este limitado efecto terapéutico no fué observado más que con los regímenes que producían reacciones tóxicas. Bacteriológicamente, la cicloserina pareció ser más netamente eficas; la infecciosidad viró en 30 (45 por ciento) de 66 enfermos puestos a cicloserina y sólo en 4 (21 por ciento) de 19 tratados con placebos.

La cicloserina produjo reacciones tóxicas graves en 18 de 115 enfermos. Acusó cierta eficacia terapéutica según indican la falta de deterioro roentgenográfico y un coeficiente moderadamente alto de viraje bacteriológico. La excelencia de otras disponibles combinaciones antimicrobianas y la toxicidad de la cicloserina probablemente vedarán su empleo para tratamiento inicial. No obstante, debe determinarse con ensayos ulteriores su utilidad a dosis pequeñas, ya sola o en una combinación terapéutica, para los enfermos que no han respondido adecuadamente a combinaciones de isoniacida, estreptomicina y PAS.

RESUME

Etude-pilote de la toxicité de la Cyclosérine

Une enquête pour déterminer les effets toxiques de la cyclosérine a été entreprise dans six hôpitaux chez 141 tuberculeux. Cette étude a été organisée de telle manière que ni les malades ni les médecins ne pouvaient savoir s'ils recevaient le médicament ou le "placebo." La plupart de malade étaient porteurs de lésions tuberculeuses pulmonaires très avancées et cavitaires et leur état général était très mauvais malgré une chimiothérapie intense antérieure.

Les diverses modalités d'administration de la cyclosérine étudiées étaient: 1 dose de 1,5 g deux fois par jour; 1 dose unique de 1 g tous les deux jours; 1 dose unique de 0,5 g par jour; 1 dose de 0,25 g deux fois par jour. L'examen neurologique et l'examen du liquide céphalorachidien furent pratiqués avant le traitement et au bout d'une cure de douze semaines. Dans trois des hôpitaux des électroencéphalogrammes ont été également effectués au commencement et à la fin de la période d'observation. Les malades étaient examinés chaque jour pour déceler les modifications psychiques et neurologiques. Des rapports sur l'état clinique, bactériologique et radiologique furent établis chaque mois.

Durant la cure de douze semaines, des phénomènes toxiques assez marqués pour justifier une modification du schéma thérapeutique se sont manifestés chez dix huit malades qui recevaient la cyclosérine; ils ont fait défaut chez les malades recevant le "placebo". Huit malades ont eu des convulsions; deux, des troubles mentaux; trois, des troubles moteurs; un, de la somnolence; trois, des vertiges et un, de la fièvre et des frissons. Trois des programmes d'administration de la cycloserine ont provoqué des reactions toxiques chez 11 des 25 malades recevant 0,5 g deux fois par jour; chez 5 des 39 malades recevant 1 g tous les deux jours et chez 2 des 38 malades recevant 0,5 g une fois par jour.

Bien que le programme établi pour l'administration de la cyclosérine ait du être interrompu chez 18 malades au cours des premiers trente quatre jours de traitement, aucun de

ceux qui avaient dépassé cette période de trente quatre jours sans incidents, n'a présenté de réactions d'intolérance durant le reste de la cure de douze semaines.

Il n'a été observé aucune augmentation constante de la protéine du liquide céphalo-rachidien en rapport avec le traitement par la cyclosérine ou avec les réactions d'intolérance au médicament. Les électroencéphalogrammes ne so sont pas montrés utiles pour désigner avant le traitement les malades qui ont ultérieurement présenté des manifestations toxiques, ils n'ont pas non plus révéle les altérations résiduelles apres l'apparition des manifestations de toxicité. Les examens neurologiques n'ont pas révelé d'uniformité dans les différents types de lésions.

Bien que les radiogrammes des malades traités par la cyclosérine n'aient pas révélé l'existence d'une amélioration remarquable au cours du traitement, la comparaison avec les radiogrammes des malades recevant le "placebo", indiquait que la cyclosérine semblait prévenir toute nouvelle altération. Toutefois, cet effet thérapeutique limité ne fut observé qu'avec les programmes thérapeutiques produisant les réactions toxiques. Du point de vue bactériologique, la cyclosérine apparaissait plus nettement efficace. La négativation du crachat était obtenue chez 30 (45%) des 66 malades traités par la cyclosérine et chez seulement 4 (21%) des 19 malades recevant le "placebo."

La cyclosérine a produit des réactions d'intolérance graves chez 18 des 115 malades. Elle possède un certain degré d'efficacité thérapeutique comme l'a indiqué l'absence d'aggravation des images radiologiques et par un taux de négativation bactériologique modéré. L'excellence des autres associations antimicrobiennes dont on disose et la toxicité de la cyclosérine préviendront probablement son utilisation dans le traitement initial. Il y a toutefois lieu de poursuivre les essais pour déterminer sa valeur à doses faibles, soit quand elle est administrée seule soit dans la thérapeutique combinée, chez les malades n'ayant pas répondu de façon satisfaisante aux associations d'isoniazide, de streptomycine et de PAS.

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VENTILATION IN CHRONIC PULMONARY EMPHYSEMA[1, 2]

I. Pressure-Volume and Pressure-Flow Relationships

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INTRODUCTION

Changes in the visco-elastic properties of the lung and progressive bronchial narrowing are the prominent features in the pathophysiology of chronic pulmonary emphysema (1). These changes first affect the ventilatory function of the lungs. The disturbances in alveolar-capillary gas exchange appear secondarily, and usually determine the outcome of the disease. Treatment should be aimed at the improvement of ventilatory function, thereby preventing further progress of the disease. Therapeutic attempts in this direction, however, have not been too successful. One of the reasons for this failure lies in the fact that the changes in visco-elastic properties are poorly understood and inaccessible to treatment. The therapeutic attempts, therefore, have to be centered around the bronchospastic component of the disease.

Ventilation is the first step in the chain of events leading to the fulfillment of the aerobic metabolic needs of the body. Its evaluation is necessary for the understanding of any cardiopulmonary disease. Unfortunately, the clinician is faced with a battery of ventilatory function tests whose limitations are not always realized.

In the past, great emphasis has been placed upon the different lung-volume and time-volume relationships in the bellows action of the chest. Interest in the work of breathing has been reawakened only during recent years.

It is apparent that the lung volumes have no *direct* relation to the efficiency of pulmonary function.[3] The determination of vital capacity (maximal stroke volume), maximal breathing capacity (maximal ventilatory effort over a limited time), and similar pulmonary function tests (timed vital capacity, maximal expiratory flow rate) *does not take into account the forces involved during that particular ventilatory process.* Furthermore, there usually are basic differences in the mechanics of breathing during quiet respiration and during maximal ventilatory efforts in patients with chronic pulmonary emphysema. Therefore, the results of routine function tests are not always easy to relate to ventilatory function. These differences are due mainly to highly increased expiratory flow rates, leading to ball valving (2, 3) or what might be called "bronchial chattering."

Although the routine pulmonary function tests (spirogram, vital capacity,

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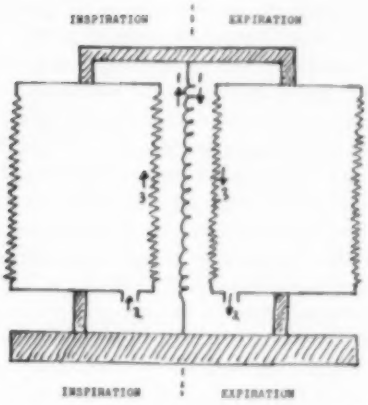
[3] This is true for all subdivisions of the lung with the exception of the mid position. The latter influences both compliance and mechanical resistance.



Forces necessary to move air in and out of the lungs:

A. Elastic force; a function of the lung volume (lung distention) analogous to an elastic spring (1)

B. Force necessary to overcome mechanical resistance, a function of the speed at which the air travels.
   a. Resistance to air flow (2)- air flowing through the nozzles of the bellows.
   b. Resistance to tissue deformation (3)- deformation of the fabric of the bellows.

FIG. 1. Schematic presentation of lung forces involved in the breathing mechanism.

timed vital capacity, maximal breathing capacity, and the variously derived ratios) are of help to the clinician, they do not always contribute greatly to the pathophysiologic evaluation of the cardiopulmonary patient. Moreover, correlation with the degree of actual incapacity is often poor.

THE WORK OF BREATHING

It must be emphasized that minute ventilation bears no relation to the work of breathing as long as the pressure components are not considered. If one considers the lungs as a pair of bellows (figure 1) connected with a handle (functioning analogous to the intrapleural space) and fixed by an elastic spring, three different forces are acting between the lungs and the intrapleural space. These forces are balanced by the sum of the thoracic forces: the muscular forces of the chest wall, the elasticity of the chest wall, and the viscosity of the chest wall. The spring represents the elasticity of the lung tissue; the fabric of the bellows depicts the viscosity of the lung tissue; and the openings in the bellows portray the airways. If one pulls the handle, the bellows are distended and air enters as long as the distention is continued.

During this procedure, the elastic force of the spring and the mechanical resistance of the bellows system, namely, resistance to deformation (viscosity) and to air flow, have to be overcome. Once the bellows are distended and one lets the handle go, they will collapse provided the elastic recoil of the spring is sufficient to overcome the mechanical resistance of the system (passive expiration). If the spring is too weak, the handle has to be pushed in order to overcome tissue viscosity and air flow resistance. It is apparent that either a decrease in elastic recoil or an increase in mechanical resistance can lead to the same result, namely, an active expiration.[4]

[4] This analogy does not hold true in all aspects, due to the fact that the lung has volume elastic properties, while the elasticity of a spring is a function of its linear distention.

Compliance: The elastic properties of the lungs can be expressed as compliance, which is defined as the volume change per unit of pressure change (L per cm. H_2O). A lung with a low compliance is considered stiff and requires a greater force for its distention than a lung with a higher compliance. Compliance is a linear function of volume in the normal lung within the normal breathing range (4). However, if there is unequal ventilation of the lung, the distention of different lung regions will vary. The volume of air they receive will differ also. The equilibration of alveolar pressure will depend upon the time available for each breath. The higher the respiratory rate, the more marked the unequal ventilation. During high respiratory rates, only the normal alveoli will be adequately ventilated; the others will have a reduced ventilation. Therefore, a higher pressure difference will be necessary for a given volume change, and the compliance drops with the increase in respiratory rate. If the respiratory rate is sufficiently small, the intrapulmonary pressure differences between unequally ventilated alveoli will equilibrate at the end of each inspiration and expiration, and the compliance will rise.

Resistance: Mechanical resistance expresses the pressure necessary for a given air flow, and is measured as cm. H_2O per L per sec. This pressure depends upon the viscosity of the lung tissue, the properties of the inspired gases, and the structure of the bronchial tree. The resistance due to tissue viscosity has been measured to be 30 to 40 per cent of the total mechanical resistance in normal lungs (5). The contribution of tissue viscosity to resistance in diseased lungs is unknown. Compliance and mechanical resistance, similar to all other ventilatory measurements, change with body position. The present writers have noted this in both normal persons and patients with cardiopulmonary disease (6).

Pressure gradient: A pressure gradient is necessary in order to produce air flow. The air flows from a high pressure area to a low pressure area (figure 2). During inspiration, the distention of the thorax creates a negative intrapulmonary pressure. During expiration the conditions are reversed. In the bronchial tree, the pressure (pc) falls in the direction of the air flow while the surrounding alveoli are exposed to a uniform pressure (palv). If the pressure drop within the bronchial tree becomes too large because of high flow rates, the elastic recoil of the lung (pd) may be unable to maintain patency of the airways. They may then collapse because of the high positive intrapulmonary pressure during an expiratory effort (figure 2*C*). At this moment the air flow will decrease rapidly until the intrabronchial pressure rises to a point at which it counterbalances intrapulmonary pressure. Air flow resumes and then the vicious cycle starts again. The precise moment in which this ball valve or check valve mechanism occurs depends upon the intrapulmonary pressure, the air flow rate, the lung elasticity, and the structure of the bronchial and peribronchial tissues.

Emphysema: The above mechanism occurs frequently in chronic pulmonary emphysema. It is not always readily recognized in the conventional pulmonary function tests which place great emphasis upon time-volume relationships (timed vital capacity, maximal breathing capacity, et cetera). The applied expiratory force is twofold: first, the elastic recoil which is a function of the lung distention;

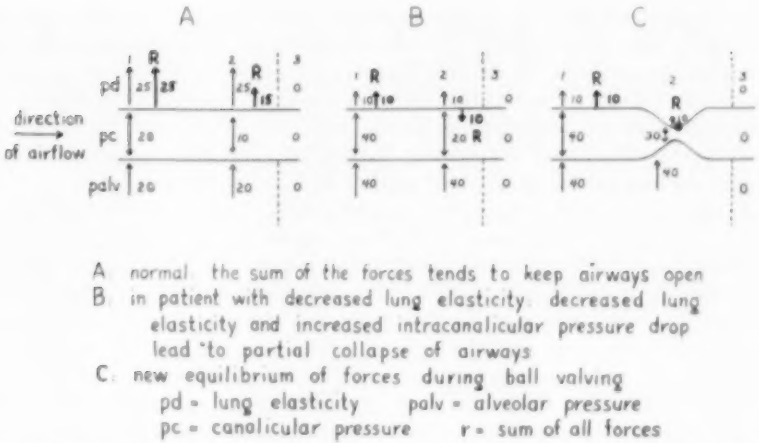

A
B
C
direction of airflow
pd
pc
palv
R
A normal the sum of the forces tends to keep airways open
B in patient with decreased lung elasticity decreased lung elasticity and increased intracanalicular pressure drop lead to partial collapse of airways
C new equilibrium of forces during ball valving
pd = lung elasticity palv = alveolar pressure
pc = canalicular pressure r = sum of all forces

FIG. 2. Schematic diagram of the pressure relations in the airways at three different levels during forced expiration: (1) terminal bronchioles, (2) bronchial tree, and (3) oropharynx.

and, second, the force applied by the expiratory muscles. The elastic recoil is sufficient to expel the tidal volume in a normal lung and also tends to maintain the patency of the airways (figure 2). During timed vital capacity maneuvers in which large volumes are moved within a short period of time, the elastic force is a minor factor in the development of initially high flow rates. However, a decrease of the elastic force plays a considerable role in the development of the ball valve mechanism. Therefore, in patients with chronic pulmonary emphysema, smaller expiratory pressures may lead to higher mean flow rates and higher expired volumes because ball valving is avoided to some degree. For this reason, the time-volume relationship seems to be of little value in patients with chronic pulmonary emphysema, as the volume expired per unit of time depends upon the moment at which leakage flow develops. This moment is determined by the weakening of the tracheobronchial and peribronchial structures, the elastic recoil of the lung, and the pressure gradient developed from the pleura to the oropharynx. Leakage flow manifests itself readily in the expiratory air flow pattern by notches and less noticeably by angles in the descending limbs of the spirogram (figure 3*A*). The recording characteristics of the currently used spirometers are often inadequate under these circumstances (7).

Comparison of the mechanics of breathing in a normal subject with a patient with chronic bronchial asthma and one with chronic pulmonary emphysema: Some of the characteristics discussed above are shown in figures 3*A* to *D*, in which the mechanics of breathing in a normal patient, a patient with chronic bronchial asthma (after induction of bronchospasm by the injection of methacholine, 0.1 mg. subcutaneously), and a patient with severe chronic pulmonary emphysema are compared. In the top tracing (figure 3*A*), the volume changes are recorded;

in the second tracing, the venous pressure in the first two subjects and the arterial pressure in the patient with chronic pulmonary emphysema are shown. The influence of changes in the intrathoracic pressure on the circulatory dynamics can readily be seen even during quiet breathing in patients with pulmonary disease. The venous pressure rises during the period in which the intrathoracic pressure is positive, and the arterial pressure and the pulse pressure decrease during the inspiratory phase and recover during the expiratory phase. The third tracing records the changes in the intraesophageal pressure during inspiration and expiration. The drawn pencil lines connect the points of zero flow rates and have been corrected for the volume changes per unit time. They represent static intrapleural pressures; namely, the elastic recoil of the lung at that given volume. The difference between the static and dynamic (actual) intrapleural pressure represents the resistive pressure necessary to obtain the flow rate at that particular moment. The flow rates are shown on the bottom tracing.

It will be seen that the patient with chronic bronchial asthma or pulmonary emphysema produces lower flow rates, requires a greater pressure difference to move air in and out of the lung, and needs proportionally more time for expiration than the normal subject (figure 3*A*).

The pressure-volume relationships in these three patients have been plotted (figure 3*B*) from the data obtained in the recordings noted in figure 3*A*. The normal subject forms a narrow loop. The diagonal line represents the compliance and forms a steep angle with the volume axis (normal compliance). It will be noted that the loops in the patients with chronic bronchial asthma and chronic pulmonary emphysema are much wider. This indicates that most of the work of breathing goes towards overcoming the mechanical resistance and only a small part towards overcoming the elastic forces of the lung. Furthermore, the elastic recoil of the lung is insufficient to perform the expiration. The increased resistance has to be overcome by an active effort of the expiratory muscles.

It will be noted that intraesophageal pressures are positive during the major part of expiration. As a rule, absolute intraesophageal pressures are more positive than absolute intrapleural pressures. This difference depends upon the following factors: the position and inflation of the esophageal balloon, the body position, and the physical characteristics of the mediastinal structures. Therefore, it is usually impossible to determine from esophageal pressures either the mid position or the absolute values for the work of breathing. The latter includes the metabolic work to keep the lung distended at its mid position.

Although the absolute pressures in the esophagus and the intrapleural space may be quite different, the pressure changes during the respiratory cycle are about equal at the two different sites *under proper experimental conditions* (6). Therefore, values for compliance and mechanical resistance are equal and independent of the site of measurement.

In figure 3*C*, the resistive pressures (x axis) have been plotted against the flow rates (y axis). The curves represent the mechanical resistance obtained in these three subjects. The two patients show a marked increase in resistance during both inspiration and expiration. The expiratory tracing shows a loop instead

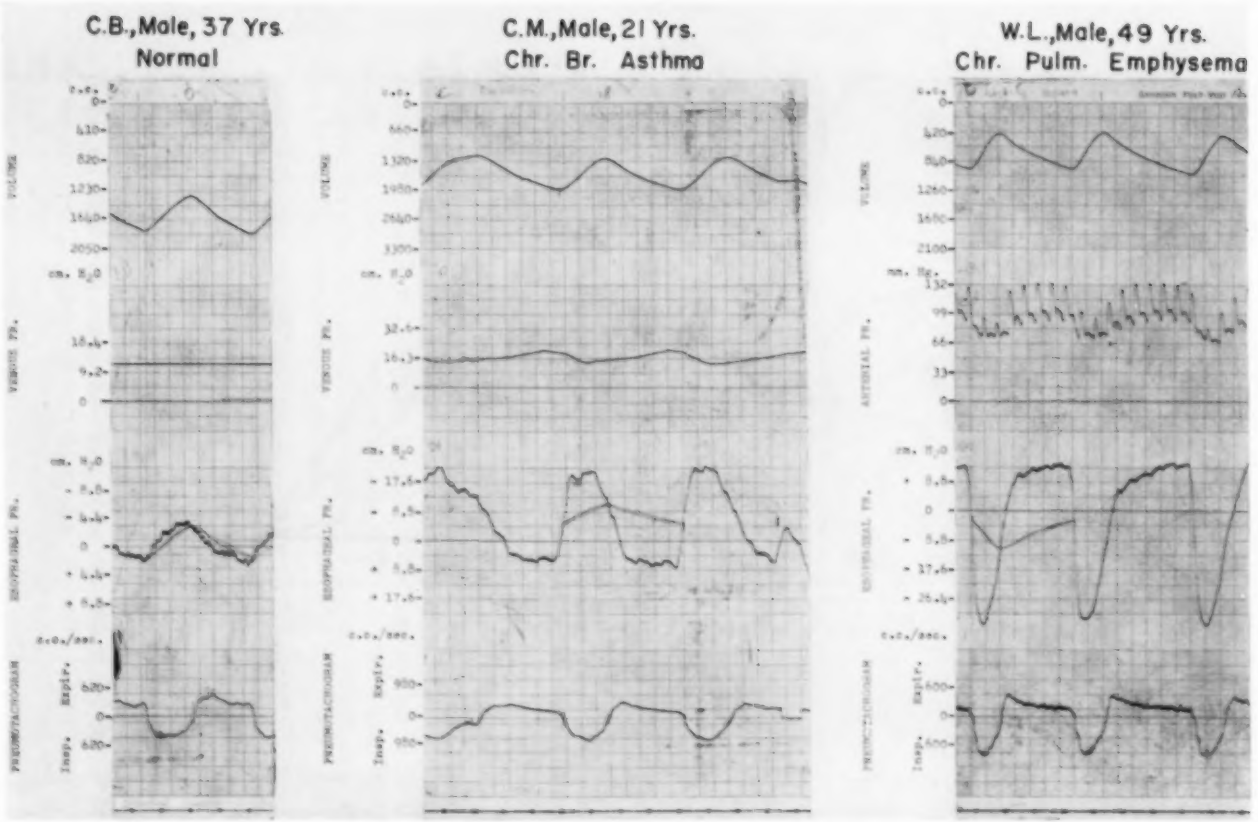


FIG. 34. Ventilatory dynamics in supine position during quiet breathing in a normal patient, a patient with chronic bronchial asthma during induced bronchoconstriction, and a patient with severe chronic pulmonary emphysema.

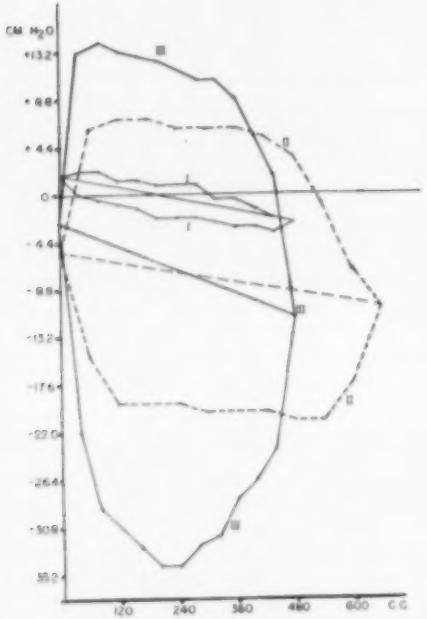


FIG. 3B. Pressure-volume relationship during quiet breathing in normal patient (I); in patient with chronic bronchial asthma during induced bronchoconstriction (II); and in patient with long-standing severe chronic pulmonary emphysema (III) (supine position).

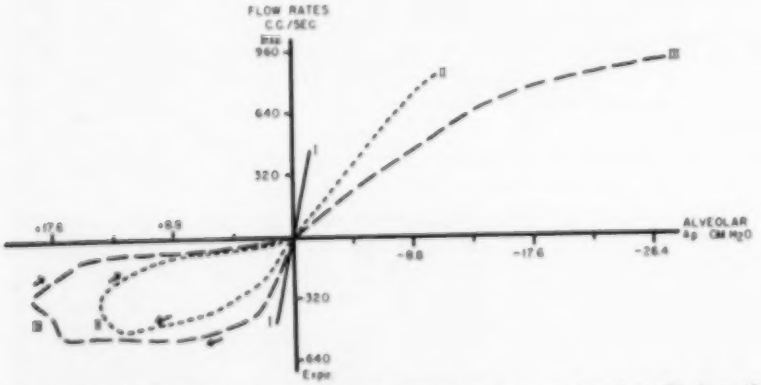


FIG. 3C. Mechanical resistance during quiet breathing in normal patient (I); in patient with chronic bronchial asthma during induced bronchoconstriction (II); and in patient with severe long-standing chronic pulmonary emphysema (III) (measurements in supine position).

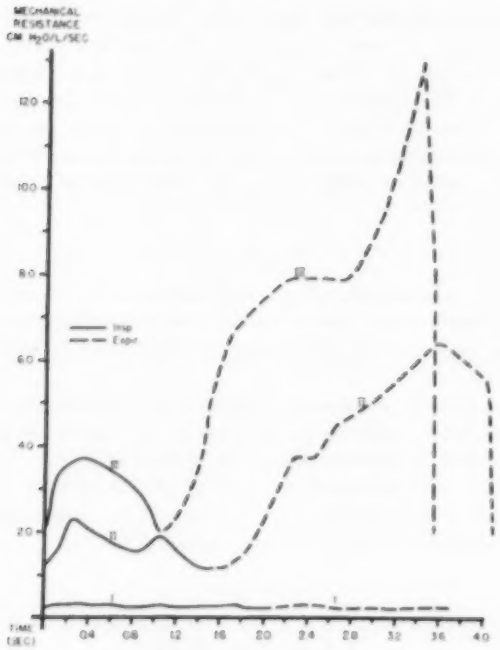


FIG. 3*D*. Mechanical resistance-time relationship in different patients in supine position during quiet breathing: I. normal; II. chronic bronchial asthma; III. chronic pulmonary emphysema.

of a single line as noted in the normal subject. This is due to ball valving. The pressure necessary to obtain a given flow rate increases progressively during the expiratory cycle.

In figure 3*D*, mechanical resistance has been plotted against time in the three subjects. The normal subject shows about the same resistance during the whole cycle, while the other two patients have a marked increase during inspiration[5] and a tremendous increase during expiration.

DISCUSSION

The studies presented in figures 3*A* to *D* clearly demonstrate why patients with chronic bronchial asthma or chronic pulmonary emphysema can be fully incapacitated, despite very little roentgenographic or pathologic evidence of disability. A large part of their energy and their oxygen uptake is spent on the breathing process alone.

Although Rohrer (8) introduced the modern concept of the mechanics of

[5] The increase in mechanical resistance during inspiration has not been taken into consideration in the development of the various function tests which have been designed to replace the maximal breathing capacity.

breathing forty years ago, the full significance of pressure-volume and pressure-flow relationships has only been recognized in the past few years. There are still large gaps in our knowledge of the physical properties of the human lung which remain to be solved. The clinician should realize that an evaluation of ventilatory function on the basis of volume measurements or volume-time relationships alone may be as incomplete and as misleading as evaluating cardiac function on the basis of cardiac output without taking into consideration blood pressures and vascular resistance.

SUMMARY

The importance of pressure-volume and pressure-flow relationships in the evaluation of patients with chronic pulmonary emphysema is stressed. The limitation of the conventional pulmonary function tests in this report is discussed.

Patients with chronic pulmonary emphysema produce lower flow rates, require greater pressure gradients to move air in and out of the lung, and need proportionally more time for expiration than normal subjects.

Loss of elasticity and increased pressure gradients lead to expiratory ball valving, especially under conditions of stress, e.g., hyperventilation, vital capacity, and maximal breathing capacity maneuvers.

SUMARIO

La Ventilación en el Enfisema Pulmonar Crónico: I. Relaciones del Volumen-Presión y de la Corriente-Presión

Recálcase la importancia que poseen las relaciones del volumen-presión y de la corriente-presión en la justipreciación de los sujetos que padecen de enfisema pulmonar crónico. Discútese la limitación que rodea en este sentido a las pruebas corrientes de la función pulmonar.

Los enfermos con enfisema pulmonar crónico muestran índices más bajos de la corriente de aire, requieren pendientes más altas de la presión para introducir y sacar el aire de los pulmones y necesitan proporcionalmente más tiempo que los sujetos normales para la espiración.

La pérdida de la elasticidad y las pendientes más altas de la presión conducen a efectos de válvula de bolas en la espiración, sobre todo en condiciones de esfuerzo, v.g., maniobras relacionadas con la hiperventilación, la capacidad vital y la máxima capacidad respiratoria.

RESUME

Ventilation dans l'emphysème pulmonaire chronique: I. Rapports entre volume-pression et débit-pression

L'importance des rapports pression-volume et pression-débit dans l'évaluation des cas d'emphysème pulmonaire chronique est mise en évidence. La limitation des tests conventionnels de la fonction pulmonaire est étudiée dans ce rapport.

Chez les malades atteints d'emphysème pulmonaire chronique les taux de débit sont plus faibles, les gradients de pression réclamés pour la pénétration et le rejet de l'air dans le poumon sont plus élevés et ces malades ont proportionnellement besoin de plus de temps pour l'expiration que les sujets normaux.

La perte d'élasticité et l'accroissement des gradients de pression conduisent au "*ball-valving*" expiratoire, en particulier dans les conditions de stress, c'est à dire dans l'hyper-

ventilation et les manoeuvres utilisées dans la détermination de la capacité vitale et de la capacité respiratoire maxima.

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VENTILATION IN CHRONIC PULMONARY EMPHYSEMA[1, 2]

II. Correlation of Compliance and Mechanical Resistance with Routine Pulmonary Function Tests

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INTRODUCTION

In the first paper of this series (1), the importance of pressure-volume and pressure-flow relationships in the evaluation of patients with chronic pulmonary emphysema was discussed. The purpose of the present study is to report the results in 12 patients with chronic pulmonary emphysema in whom the mechanics of breathing were correlated with the conventional pulmonary function tests.

MATERIAL AND METHODS

Twelve patients with an established diagnosis of chronic pulmonary emphysema were studied. Vital capacity and subdivisions of the lung were measured with a 9 liter Collins spirometer; maximal breathing capacity, with a low resistance Rudolph-valve and a Douglas bag; and residual volume and intrapulmonary mixing, by the seven-minute nitrogen washout method (2). The predicted values were derived by using Baldwin's formulas. These tests were carried out in sitting position.

Arterial blood was collected after a resting period in the supine position from the brachial artery and analyzed for carbon dioxide and oxygen content by the method of Van Slyke and Neill. The pH was determined with a Cambridge Research Model pH Meter and pCO_2 was calculated using the nomogram of Singer and Hastings. Intraesophageal pressures were substituted for intrapleural pressures. These were measured by an intraesophageal tube with multiple holes at its distal end which were protected by a thin-walled latex balloon of 15 cm. in length. The balloon was placed in the lower third of the esophagus and was kept in a nearly deflated state (inflation by 1.0 ml. of air). The tube was connected to one side of a differential transducer. The other side of the transducer was connected to the mouthpiece. Air flow rates were measured with a 400 Monel mesh 3 square inch pneumotachograph,[3] and the volumes were obtained by electrical integration of the flow rate signal. Appropriate correction was made for volume changes between inspired and expired air due to variation in temperature and humidity.

All pressures were recorded through mechano-electrical transducers on a four-channel Sanborn polyviso. Two series of ten breaths each were analyzed and the mean compliance of the two series had to check within 0.01 for values below 0.1, and within 0.02 for values between 0.1 and 0.15. Mechanical resistance was obtained by dividing the resistive pressure by the flow rate at intervals of 0.1 seconds for slow respiratory rates and at intervals of

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[1] From the Department of Medicine, Tufts University School of Medicine, and Lung Station (Tufts) and Department of Inhalation Therapy, Boston City Hospital, Boston, Massachusetts.

[2] This study was made possible by a grant-in-aid from the Tobacco Industry Research Committee, New York, New York.

[3] This apparatus measures the pressure difference across a fine wire screen and gives a linear response over the range of respiratory flows encountered during most respiratory experiments.

0.04 seconds for fast respiratory rates. The mean mechanical resistance was determined planimetrically by plotting mechanical resistance against time. These studies were done in the supine position, where compliance values are approximately 25 per cent lower and resistance values approximately 20 per cent higher than in the sitting position (3). Both slow and fast respiratory rates were studied whereby the range was selected by the patient.

RESULTS

The results of all of the function tests are summarized in table 1, together with the physical characteristics of the patients. Three patients had a reduction of the vital capacity below 75 per cent of the predicted value, while only 2 patients had a maximal breathing capacity within the expected values. In 7 of 11 patients, the air velocity index was significantly reduced. An increased residual volume-total lung capacity (RV/TLC) ratio, unequal ventilation, and poor intrapulmonary mixing were present in all instances. With the exception of one patient, all patients showed arterial oxygen unsaturation at rest. The pCO_2 was high in 3 patients and the pH was low in one patient.

The compliance values showed a wide range of variation at basal respiratory rates but, in all but one case, there was a marked drop when the respiratory rate was increased (figure 1*C*). The one patient who did not show this drop had a very low initial compliance value. Mechanical resistance during inspiration was high in every instance, varying from 1.2 to 14 times the normal (average 4.25 times normal). This increase was even more marked during expiration (2.2 to 25 times normal, average 6.6 times normal). These findings corroborate the results of Fry and associates and of Mead and co-workers (4, 5).

DISCUSSION

The usual findings in chronic pulmonary emphysema are increased residual volume, poor intrapulmonary mixing, and decreased maximal breathing capacity. The vital capacity is usually only slightly reduced (2). Changes in arterial blood gases depend upon the amount of ventilatory disturbance. These findings are confirmed in the present study and correlated with the observed changes in compliance and mechanical resistance.

In normal patients and in patients with cardiac failure, there is good correlation between vital capacity and compliance (6–8). This correlation is much poorer in patients with chronic pulmonary emphysema (figure 1*A*). This is understandable, as changes in the visco-elastic properties of emphysematous lungs lead to a decrease in elasticity and unequal ventilation. Consequently, the correlation between compliance and intrapulmonary mixing index is much better in the patients of the present study (figure 1*C*). In this figure, the alveolar nitrogen concentration is plotted against the compliance values obtained at slow and fast respiratory rates.

The patients with poor intrapulmonary mixing, in general, show a low compliance. However, in their present form, neither the nitrogen washout method nor the determination of compliance are ideal methods for the determination of intrapulmonary mixing. The nitrogen washout method tends to give low alveolar nitrogen values in hyperventilating subjects. The compliance value

TABLE 1

PULMONARY FUNCTION TESTS IN TWELVE PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA

Name	Sex	Age	BS	VC	Predicted VC	MBC	Predicted MBC	AVI	RV	RV/TLC	Intrapulmonary Mixing	Compliance Supine Position	Inspiration Mechanical Resistance Supine Position	Expiration Mechanical Resistance Supine Position	Arterial Saturation	pH	pCO_2
			m^2	*ml.*	*ml.*	*L/min.*	*L/min.*		*ml.*	*per cent*	N_2 *per cent*	*L/cm. H_2O*	*cm. H_2O/ L/sec.*	*cm. H_2O/ L/sec.*	O_2 *per cent*		*mm. Hg.*
W.L.	M	49	1.99	1,900	3,000	—	—	—	4,800	65.0	12.0	0.028	38.8	41.8	76.5	7.42	50.0
J.S.	M	55	1.77	3,410	3,590	60.5	102.0	0.62	3,950	53.0	7.8	0.070	10.1	48.0	84.0	7.41	44.0
A.R.	M	61	1.53	3,320	3,450	32.0	84.0	0.40	4,490	57.0	14.0	0.139	10.2	15.2	86.5	7.40	43.0
W.G.	M	61	2.07	2,690	2,960	49.6	113.0	0.48	4,270	56.5	3.1	0.132	9.0	9.0	88.5	7.39	35.5
A.B.	M	61	1.76	3,170	3,600	99.5	101.0	1.12	2,920	46.0	6.0	0.100	5.3	9.6	85.7	7.40	43.0
M.N.	M	50	1.68	3,000	3,530	51.0	101.0	0.59	5,620	64.0	9.7	0.120	9.4	13.8	86.0	7.40	44.0
J.S.	M	48	1.61	2,720	3,360	50.0	99.0	0.62	4,320	59.0	15.0	0.067	8.0	8.0	87.6	7.40	44.0
A.DeW.	F	51	1.56	2,300	2,660	58.0	73.5	0.92	2,210	49.0	6.2	0.050	12.2	19.3	97.0	7.42	39.0
E.J.	M	63	1.72	1,530	2,720	52.3	92.0	1.00	5,780	74.0	19.6	0.058	6.8	10.7	81.4	7.41	39.0
B.B.	M	65	1.67	3,560	5,000	35.2	88.0	0.57	2,530	50.0	13.5	0.045	13.0	13.6	82.0	7.38	50.0
W.F.	M	73	1.56	2,760	3,120	65.4	75.5	1.00	2,600	47.0	3.5	0.145	3.2	6.8	88.6	7.39	37.0
B.G.	M	63	2.04	2,670	3,550	42.6	109.0	0.52	3,440	51.0	13.2	0.050	14.3	14.5	73.5	7.30	84.0
Mean		58		2,760	3,380	54.0	94.5	0.71	3,920	56.0	10.3	0.084	11.7	17.5	85.0	7.38	46.0
Normal values for this age group				3,380		94.5		1.00	2,430	40.0	<2.5	0.140	2.75	3.1	95.0	7.40	40.0

BS = body surface area
VC = vital capacity
MBC = maximal breathing capacity
AVI = air velocity index
RV = residual volume

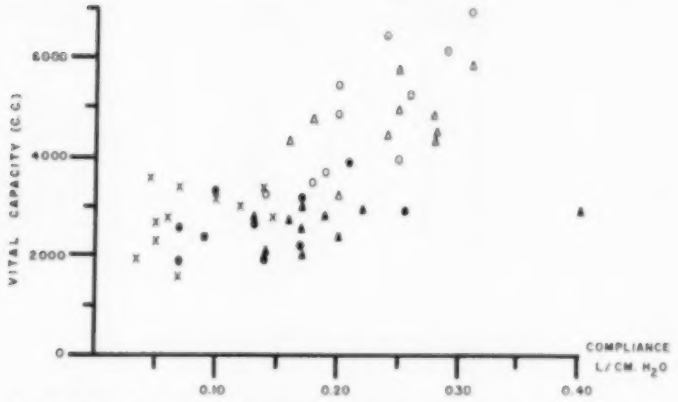

FIG. 1A. Relation between vital capacity and compliance in:
△ 10 normal subjects (Stead *et al.*)
○ 10 normal subjects (Mead *et al.*)
▲ 10 patients with chronic pulmonary emphysema (Stead *et al.*)
● 9 patients with chronic pulmonary emphysema (Mead *et al.*)
× 12 patients with chronic pulmonary emphysema (Attinger *et al.*)

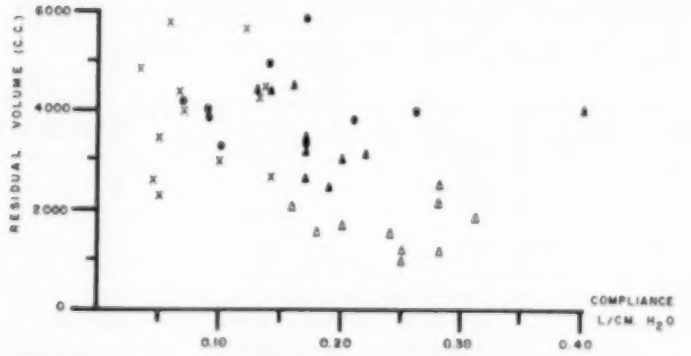

FIG. 1B. Relation between residual volume and compliance in:
△ 10 normal subjects (Stead *et al.*)
▲ 10 patients with chronic pulmonary emphysema (Stead *et al.*)
● 9 patients with chronic pulmonary emphysema (Mead *et al.*)
× 12 patients with chronic pulmonary emphysema (Attinger *et al.*)

in patients with unequal ventilation depends upon the respiratory rate which is usually increased in these subjects. Therefore, the compliance value for a given patient could be reduced during his "normal" breathing. It might be considerably higher if he could establish a respiratory rate of two or three. Inasmuch as he is unable to do this, the determination of a time independent ("static") compliance is impossible. In patients with a reduced compliance at slow respiratory rates,

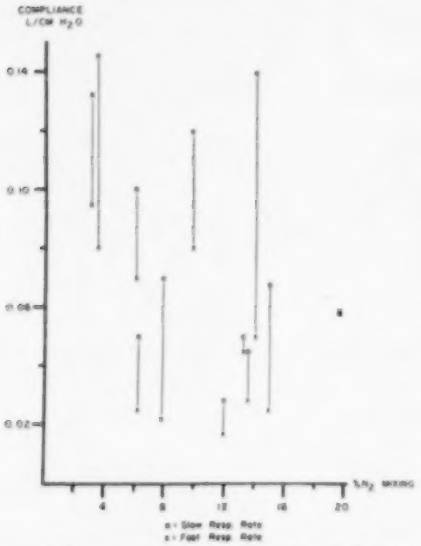

FIG. 1C. Relation between intrapulmonary mixing and compliance at slow respiratory rates and fast respiratory rates in 12 patients with chronic pulmonary emphysema.

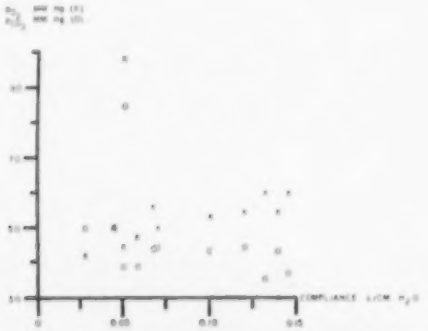

FIG. 1D. Relation between compliance and arterial pO_2 or pCO_2 in 12 patients with chronic pulmonary emphysema.

the significance of further drop in compliance with an increase in respiratory rate is very difficult to evaluate.

Neither the maximal breathing capacity nor the residual volume-total lung capacity ratio (RV/TLC) showed a correlation with compliance. The patients with a high residual volume did tend to have a low compliance in accordance with the changes in the pressure volume diagram due to the elevated mid position (7) (figure 1*B*). An interesting relationship seems to exist between compliance and arterial pO_2 (figure 1*D*). The patients with lowest compliance had

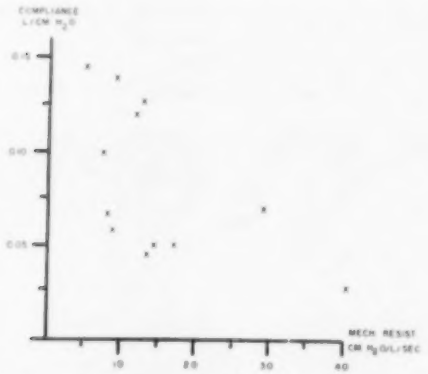

FIG. 1*E*. Relation between compliance and mean mechanical resistance of the entire respiratory cycle in 12 patients with chronic pulmonary emphysema.

the lowest pO_2. The relationship between compliance and pCO_2 was less striking, possibly because most of the patients studied had a pCO_2 in the normal range. In general, high resistance values went together with low compliance values (figure 1*E*). However, the scatter of the correlations was very wide and did not allow any general conclusion to be drawn from one measurement to another. This is understandable when one considers the factors which determine the result of a particular ventilatory function test and the complex pathophysiology of the disease.

If one regards changes in visco-elastic properties and bronchial obstruction as the basic disturbance of chronic pulmonary emphysema (figure 2), then the first disturbance would be hyperinflation and unequal ventilation, leading to an increased work of breathing. This would be manifested by changes in compliance and mechanical resistance, increased residual volume, and poor intrapulmonary mixing. As a result of the two basic factors and the unfavorable position of the inspiratory muscles, the maximal breathing capacity would be more or less affected. This first stage may lead to hypoxemia and unequal perfusion and later to the third stage with alveolar hypoventilation. Hypoxia and carbon dioxide retention lead to constriction of the pulmonary veins and venules, thereby interfering with the outflow of blood from the lungs (9). This can lead to a decrease in compliance.

In addition, a large increase of carbon dioxide has been found to effect bronchoconstriction, which also tends to decrease compliance (10). However, other investigators, using somewhat lower concentrations of carbon dioxide, have described bronchodilator effects of the increased carbon dioxide tensions (11, 12). Hypoxia and hypercapnia result from alveolar hypoventilation and may lead to pulmonary hypertension (13, 14).

On the other hand, destruction of alveolar walls leads to a restriction of the capillary bed and subsequently to pulmonary hypertension. The latter is anatomically fixed and irreversible; the blood flows at an increased rate through the

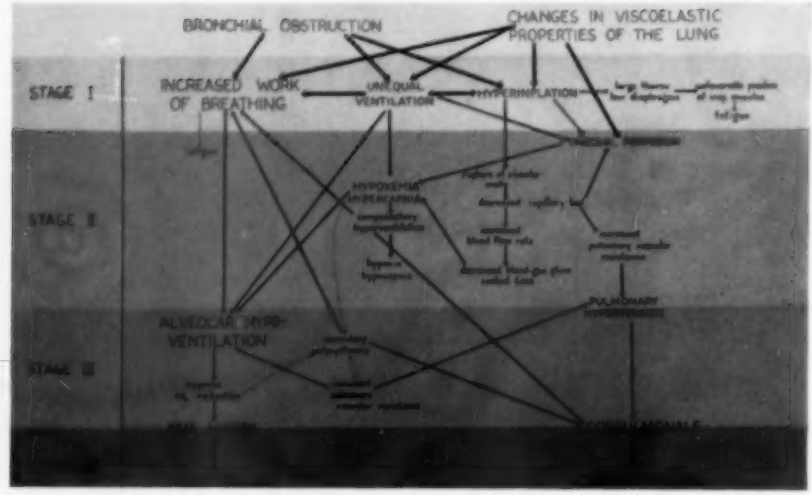

FIG. 2. Pathophysiology of chronic pulmonary emphysema. Development and degree of cardiopulmonary failure.

pulmonary capillaries, thereby decreasing the time of contact between blood and gas phase as long as the cardiac output does not fall (14). The resulting anoxemia is partially compensated by hyperventilation, thereby lowering the pCO_2. Pulmonary hypertension on the basis of alveolar hypoventilation may be reduced by relieving anoxia and/or hypercapnia as long as there are no anatomic changes of the pulmonary vessels. Finally, the fourth stage is reached: on the one hand, alveolar hypoventilation leading to respiratory acidosis and respiratory failure; on the other hand, pulmonary hypertension leading to cor pulmonale and cardiac failure.

Unfortunately, the physician usually recognizes the patient with chronic pulmonary emphysema in an advanced stage. The clinical diagnosis in the early stages can be very difficult because the patient might not present any signs or symptoms. *Measurements of compliance and mechanical resistance reveal abnormalities when the usual pulmonary function tests are still within normal range.* Chronic pulmonary emphysema is a very common and crippling disease and seems to be on the increase. The application of preventive measures and vigorous management in the early stages offers more hope than the treatment in the advanced stages. In preliminary experiments, it has been found that aminophyllin, bronchodilators, and intermittent positive-pressure breathing on inspiration can increase compliance and decrease mechanical resistance, with subsequent improvement in pulmonary gas exchange.

SUMMARY

The results of ventilatory function tests in 12 patients with chronic pulmonary emphysema are presented. These were correlated with the mechanics of breathing

(compliance and mechanical resistance) and also with the pulmonary gas exchange.

Patients with chronic pulmonary emphysema show in general a decrease in compliance and an increase in mechanical resistance. The relationship between these changes and vital capacity, residual volume, maximal breathing capacity, unequal ventilation, anoxia, and hypercapnia is briefly discussed. In general, patients with a high residual volume, poor intrapulmonary mixing, and low pO_2 show a low compliance. Unequal ventilation is manifested by a drop in compliance with an increase in respiratory rate.

The work of breathing has a significant influence on the incapacity of these patients and is an important factor in determining the degree of disability.

SUMARIO

La Ventilación en el Enfisema Pulmonar Crónico. II. Correlación de la Ejecución y la Resistencia Mecánica con las Pruebas Corrientes de la Función Pulmonar

Preséntanse los resultados de las pruebas de la función ventiladora en 12 enfermos que tenían enfisema pulmonar crónico, correlacionándose los mismos con la mecánica de la respiración (ejecución y resistencia mecánica) y además con el intercambio pulmonar de gases.

Los enfermos que tienen enfisema pulmonar crónico muestran en general disminución de la ejecución y aumento de la resistencia mecánica. Discútese sucintamente la relación entre esas alteraciones y la capacidad vital, el volumen residual, la capacidad respiratoria máxima, la desigualdad en la ventilación, la anoxia y la hipercapnia. En general, revelan mala ejecución los sujetos con alto volumen residual, defectuosa mezcla intrapulmonar y pO_2 bajo. La desigualdad en la ventilación se traduce por baja de la ejecución con aumento de la velocidad respiratoria.

Recálcase la principal consecuencia de la tarea respiratoria sobre la incapacidad de estos enfermos, señalándose la importancia que reviste en la determinación del grado de incapacidad. Discútense las limitaciones que rodean en este sentido a las pruebas corrientes de la función ventiladora.

RESUME

La ventilation dans l'emphysème pulmonaire chronique: II. Corrélation entre la résilience et la résistance mécanique par les tests classiques de la fonction pulmonaire

Les résultats des tests de la fonction de ventilation chez 12 malades atteints d'emphysème pulmonaire chronique sont présentés. Il a été établi une corrélation entre ces résultats et le mécanisme de la respiration (résilience et résistance automatique) et également avec les échanges gazeux pulmonaires.

Chez les malades atteints d'emphysème pulmonaire chronique il y a en général une réduction de la résilience et une augmentation de la résistance mécanique. Les rapports entre ces modifications et la capacité vitale, le volume résiduel, la capacité respiratoire maxima, la ventilation inégale, l'anoxie et l'hypercapnie sont brièvement étudiés. En général, dans les cas où le volume résiduel est élevé, les échanges gazeux intrapulmonaire médiocre et le pO_2 faible, la résilience est réduite. L'inégalité de la ventilation se manifeste par une diminution de la résilience et un accroissement de la vitesse des mouvements respiratoires.

L'importance primordiale du mécanisme de la respiration comme cause d'invalidité chez ces malades est mise en évidence. Les auteurs signalent l'importance de cette méthode pour déterminer le degré d'invalidité. Les limitations des tests classiques de la fonction de ventilation à cet égard sont commentées.

Acknowledgment

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TUBERCULOUS ANTIBODIES DEMONSTRATED BY AGAR DIFFUSION[1, 2]

I. Specificity and Incidence of Agar-Diffusion Antibodies in Rabbit Sera

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INTRODUCTION

There is a question of how well the serologic response which accompanies naturally occurring or experimental tuberculous infection or immunization reflects the dynamics of the host-parasite relationship. Numerous studies of the factors which influence this response have, in recent years, centered about the detection or identification of specific mycobacterial antigens from culture filtrates or extracts of bacillary masses by employing mammalian erythrocytes as adsorbents (1–6). These sensitized erythrocytes, in turn, may be used to examine sera for the presence of specific antibodies.

Despite the extensive use of these techniques in investigations of antigen-antibody reactions, the adsorptive capacity of the erythrocyte is, as yet, incompletely understood, and thus some limitation is imposed on these procedures. Since Landy (7) has reported the simultaneous or successive adsorption of several distinct bacterial antigens by the same erythrocytes, it is quite possible that the particular antigen or antibody demonstration observed in many instances is attributable only to the predominant reactants in the system being studied.

In the experiments to be described, advantage was taken of the independent diffusion and precipitation of antigens and antibodies in gels (8). Old Tuberculin was employed as antigen in an attempt to identify or separate the various precipitating antibodies in tuberculous sera. These results were then compared with those obtained by other serologic tests and with tuberculin reactivity.

MATERIALS AND METHODS

Sera: Rabbit sera: Blood samples were collected at intervals from rabbits following single intravenous injections of *Mycobacterium tuberculosis* (var. *bovis*, Ravenel), H37Rv, *M. phlei*, or BCG. The sera were removed from the clots and stored at −10°C. until needed.

In order to provide sera with high antibody titers and broad specificities, 2 rabbits were given three injections of 2.0 mg. of BCG at three-day intervals. One week following the last injection, blood was drawn and the recovered sera were stored as above.

Hemagglutination and hemolytic tests (tuberculopolysaccharides): The techniques of these tests as employed by this laboratory have been described previously (9). Essentially they are "drop" modifications of existing procedures and employ 1:15 OT[3] as the sheep cell-sensitizing antigen.

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[1] From the Department of Bacteriology, The Ohio State University, Columbus, Ohio.

[2] This investigation was supported, in part, by a grant from the Ohio Tuberculosis and Health Association.

[3] Lederle Old Tuberculin, 4X International Standard, generously provided by Dr. H. D. Piersma, Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

Sensitization of tanned cells with tuberculoproteins (6): One-tenth milliliter of washed, packed sheep cells was mixed with 1.0 ml. of 1:10,000 saline dilution of tannic acid (Mallinckrodt, Fluffy). After ten minutes' incubation in a 37°C. water bath, the cells were thrice washed with buffered saline.

Tanned PPD cells were prepared by adding 1.0 ml. of PPD[4] which contained 0.5 mg. of tuberculin per ml. After sixty minutes in a 37°C. bath, the cells were again washed three times with buffered saline and finally prepared as a 0.5 per cent suspension.

A similar quantity of tanned cells, incubated with 2.0 ml. of 1:15 OT (previously adsorbed with packed normal cells to remove tuberculopolysaccharides), was treated as above. The resulting 0.5 per cent suspension served as the second Boyden-type (protein) antigen.

PPD: The tuberculin preparations used to skin-test sensitized rabbits were the standard products manufactured by Parke, Davis and Company of Detroit, Michigan (first-, intermediate-, and second-strength doses). The tuberculins were diluted as prescribed immediately prior to testing. The same PPD employed to sensitize tanned erythrocytes for testing antibodies to tuberculoproteins on occasion served as antigen in the agar-diffusion technique.

Agar diffusion: Bacto-Agar[5] was prepared to an 0.8 per cent concentration with phosphate-buffered saline (pH 7.4) and steamed for fifteen minutes to assure complete melting.

Six milliliters of the above agar, containing 1:10,000 aqueous merthiolate, were poured into flat-bottom Petri dishes and permitted to harden at room temperature. The plates then were stored at refrigerator temperature (4°C.) until needed. Twenty minutes before testing, excess moisture was removed from the Petri dish lids with filter paper and the plates were dried at 37°C.

Filter paper discs (12.7 mm.) were placed on the agar surface, 7 mm. apart. The number of discs per plate never exceeded five and depended on the particular test being performed. Serum and antigen were placed on opposing discs by means of dropper pipettes until saturation was observed (usually 0.2 ml. sufficed). All reagents were preserved with 1:10,000 merthiolate.

The plates were incubated for two to three weeks at 4°C., during which time observations were made at three- to four-day intervals by viewing the plates in oblique light. Care was exercised to minimize temperature changes during the observation periods since the precipitates quickly disappeared at higher temperatures. This was probably due to the increased diffusion of antigen with thermal change and the ultimate dissolution of precipitate because of antigen excess. The disappearance of precipitates with time was also explained on this basis.

Frequently it was found necessary to concentrate serum samples to assure high antibody concentration. This was accomplished, as recommended by Elek (10), by pretreating the serum disc with test serum and drying *in vacuo*. Two such procedures in addition to the serum application after the disc was placed on the agar surface gave a threefold concentration.

Photography: Numerous methods were examined for achieving satisfactory lighting conditions for photographing agar diffusion plates. The most successful was the placing of the agar dish on the clear glass viewing plate of a Circline Radiograph Illuminator.[6] The surface of the viewing glass surrounding the Petri dish was masked with dark blue paper. The interior of the illuminator was similarly darkened. The oblique lighting furnished by the Circline fluorescent tube provided good contrast between the precipitates in agar and the background. Photographs were thus obtained which did not distort the appearance of the precipitates, a phenomenon which often accompanies procedures necessitating the tilting of plates to provide adequate lighting contrast.

[4] Lot X-6335-A, generously provided by the Parke, Davis and Company, Detroit, Michigan.

[5] Difco Laboratories, Detroit, Michigan.

[6] General Electric X-ray Corporation, Milwaukee, Wisconsin.

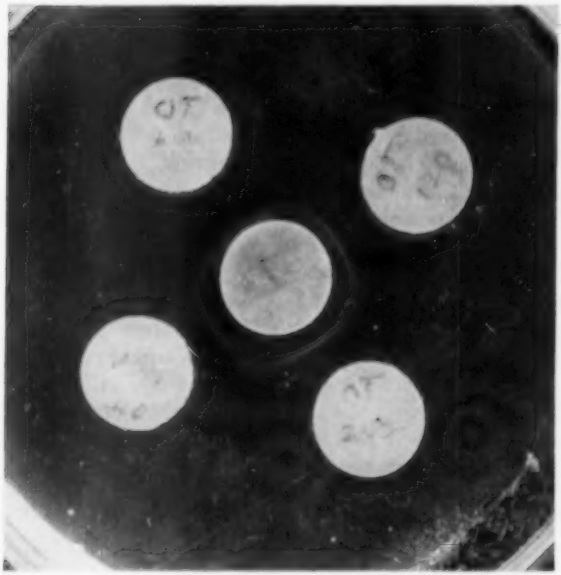

FIG. 1. Effect of antigen dilutions on position of precipitates

RESULTS

Agar Diffusion Studies with Hyperimmune BCG Serum

In order to ascertain the feasibility of applying agar-diffusion techniques to the study contemplated, an hyperimmune BCG serum (prepared in rabbits as described above) was titrated in the presence of varying dilutions of OT. A central disc saturated with undiluted serum was placed on the agar plate and surrounded by discs treated with 1:15, 1:30, 1:45, and 1:60 saline dilutions of OT, respectively. A second plate was prepared similarly, in which higher dilutions of antigen were employed (1:120, 1:240, 1:480, and 1:960). After several days' incubation, lines of precipitate were observed and the titer of this particular serum was finally determined as 1:240. The quantity and position of the precipitates were dependent in part on the concentration of antigen; the greater the concentration, the closer to the serum disc (figure 1). On occasion as many as three lines of precipitates were discernible between certain of the serum and antigen discs, suggesting multiple reactions.

Since the test sera (rabbit) were also to be examined by the sensitized-erythrocyte techniques, it was necessary to determine if heat inactivation and the removal of naturally occurring antibody by adsorption with normal sheep cells would influence the precipitating capacity of serum. The standard BCG antiserum was heated at 56°C. for thirty minutes, followed by two adsorptions with sheep cells as for serologic testing. Neither procedure appreciably altered the reactivity of the serum.

Nature of the Antigen(s) Responsible for Reaction in Agar

A series of experiments was performed to identify the antigen or antigens present in OT which precipitated the serum antibody just described.

Three milliliters of 1:15 OT were mixed with 2.0 ml. of packed, normal sheep erythrocytes for ten minutes at room temperature to remove the readily adsorbable tuberculopolysaccharides. The adsorbed OT (diluted to 1:30) still retained its antigenicity as measured against the standard serum in agar. Three additional adsorptions did not decrease its reactivity although the adsorbing cells were no longer agglutinated by a serum known to contain hemagglutinins. Thus the antigenic material was assumed to be protein or protein complexes.

Confirmation of this assumption was obtained by adsorbing 1:15 OT with tanned sheep cells. The work of Boyden (4) and Meyneli (5) has shown that tanned erythrocytes will adsorb protein as well as carbohydrate. Two milliliter quantities of tanned cells were mixed with OT as above. After four adsorptions, the OT no longer reacted with the test serum in the agar-diffusion test. Additional evidence for the protein nature of the antigens was obtained when it was shown that PPD could be substituted for OT in the agar procedure.

Specificities of Antibodies Demonstrated with Agar Diffusion

The question arose whether or not the antigens responsible for positive agar-diffusion reactions were identical with those described by Boyden and Sorkin and demonstrated with tanned erythrocytes (11).

Accordingly, the standard hyperimmune serum was tested for hemolysins and hemagglutinins with tanned cells sensitized with 1:15 OT (which had previously been adsorbed with normal cells to remove tuberculopolysaccharides) and with tanned cells sensitized with 0.5 mg. of PPD. Both antigenic preparations were used since it was believed that several protein antigens might be revealed in this manner. Although Boyden and Sorkin (11) reported demonstrating at least four protein antigens with tanned cells, their test measured only specific hemagglutinins while the present techniques would permit the detection of specific hemolysins as well.

The results indicated that the serum contained antiproteins which were demonstrated with both tanned cell antigen preparations. Hemolysins and hemagglutinins were observed with the PPD-sensitized tanned cells, while hemagglutinins alone were seen with the other test antigen.

The anti-BCG serum employed in this experiment was obtained from a rabbit which had received multiple injections of viable organisms; hence it can be assumed that it contained antibodies to minor antigens. The presence of the agar-diffusing and Boyden-type antibodies in the same serum, therefore, may be attributable to the particular method of immunization. Their coexistence does not necessarily prove that the two methods of demonstration measure antibodies with the same specificities.

The effect of time on the appearance of various antibodies following a single injection of mycobacteria has been established (12). Accordingly, the following experiment was conducted to ascertain the possible presence and relationship

TABLE 1

THE APPEARANCE OF ANTIPROTEINS FOLLOWING A SINGLE INJECTION OF MYCOBACTERIA

Serum and Week Post Injection	Tanned Cells Sensitized with Previously Adsorbed OT		Tanned PPD Cells		Agar Diffusion	
	H.L.*	H.A.*	H.L.*	H.A.*	Whole Serum	Concentrated Serum
Anti-BCG						
0	–	–	–	–	–	–
1	–	–	–	–	–	–
2	–	–	–	–	–	+
3	–	–	–	–	–	+
4	–	–	–	–	–	+
5	–	–	–	–	–	+
6	–	+	+	+	–	+
Anti-*M. bovis*						
0	–	–	–	–	–	–
1	–	–	–	–	–	+
2	–	–	–	–	+	+
3	–	–	–	–	+	+
4	–	–	–	–	–	+
5	–	–	–	–	–	+

* H.L. refers to hemolysis; H.A. refers to hemagglutination.

of these antibodies in sera procured at different time intervals from animals injected intravenously with BCG and *M. tuberculosis* (*bovis*, Ravenel).

Serum samples, collected weekly from 2 rabbits which had received 2.0 mg. of each mycobacterium respectively, were tested with the sensitized-cell suspensions as in the previous experiment. Each of the serum samples was divided into two portions, one of which was concentrated (*see* Materials and Methods), and both were examined by agar diffusion with 1:30 OT as antigen.

The experimental results (table 1) clearly illustrate the value of concentrating sera for the agar demonstrations. Under these conditions the antibodies were detectable as early as the first (anti-*M. bovis*) or second (anti-BCG) week. The failure to demonstrate antibodies to the protein-sensitized tanned cell antigens in the majority of sera suggests that either these antibodies are different from those measured in agar or that they were present in too low concentrations to be demonstrated by sensitized tanned cells.

Reactions of Randomly Selected Serum Samples

In view of these results, 31 sera were chosen at random from rabbits which had received single intravenous injections of viable BCG, *M. phlei*, H37Rv, or the bovine (Ravenel) strain. No effort was made to select sera with respect to any particular time interval following injection.

When these sera were subjected to the usual testing procedures, the same lack of correlation between agar-diffusing antibodies and Boyden-type antibodies was found as in the previous experiments (table 2). It is also apparent

TABLE 2

REACTIONS OF RANDOMLY SELECTED ANTISERA TO VARIOUS MYCOBACTERIA

Serum Samples	Tanned Cells Sensitized with Previously Adsorbed OT		Tanned PPD Cells		Agar Diffusion	
	H.L.*	H.A.*	H.L.*	H.A.*	Whole Serum	Concentrated Serum
Anti-BCG						
1	–	–	–	–	–	–
2	–	–	–	–	–	+
3	–	–	–	–	–	–
4	–	–	–	–	–	+
5	–	+	–	–	–	+
Anti-*M. phlei*						
1	–	–	–	–	–	–
2	–	+	–	–	–	–
3	–	+	–	+	–	–
4	–	+	–	+	–	+
5	–	+	–	+	–	+
Anti-H37Rv						
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	–	–	–	–	–	+
Anti-*M. bovis*						
1	–	–	–	–	–	–
2	–	–	–	–	–	+
3	–	–	–	–	–	–
4	–	–	+	–	–	–
5	–	–	+	–	–	–
6	–	–	–	–	–	+
7	–	–	–	–	–	+
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–
11	–	–	–	–	–	–
12	–	–	–	–	–	–
13	–	–	+	–	–	–
14	–	–	–	–	–	–
15	–	–	+	–	–	–

* H. L. refers to hemolysis; H.A. refers to hemagglutination.

that the two tanned cell antigens probably detected different protein antibodies in most instances in which reactions were observed.

With some of the agar-diffusion tests, in addition to concentrating the test serum, it was necessary to dilute the antigen beyond 1:30 before a visible reaction would occur. This procedure is in keeping with the suggestions of numerous other investigators who reported that discernible reactions take place only when the reactants are in equivalent proportions.

TABLE 3

AGAR-DIFFUSING ANTIBODIES AND TUBERCULIN HYPERSENSITIVITY

Intravenous Injection	Test Interval	Tuberculin Reactivity After 48 Hours (Mm).			Results of Agar-Diffusion Test
		First Strength	Intermediate Strength	Second Strength	
	days				
BCG (2.0 mg.)	4	—	—	—	—
		—	—	—	—
	9	—	—	—	+
		—	—	—	—
	17	—	3 x 3	13 x 15	+
		—	3 x 5	10 x 14	+
	27	—	—	8 x 10	+
		—	10 x 10	15 x 20	+
M. phlei (2.0 mg.)	4	—	—	—	—
		—	—	—	—
	9	—	—	—	—
		—	—	8 x 8	+
	17	—	—	—	+
		—	—	6 x 7	+
	27	—	—	8 x 10	+
M. bovis (0.25 mg.)	4	—	—	—	—
		—	—	—	—
	9	—	—	—	—
		—	—	10 x 15	—
	17	No test	No test	No test	—
		5 x 8	20 x 21	30 x 30	—
	27	6 x 9	20 x 20	30 x 30	—
		8 x 8	20 x 25	25 x 30	—

Agar-Diffusing Antibodies and Tuberculin Reactivity

The protein specificity of the agar-diffusing antibodies and their presumable independence from the antiproteins measured by the sensitized erythrocytes employed in this study suggested a series of experiments designed to determine their relationship with tuberculin hypersensitivity. Further justification for this aspect of the investigation was provided by the work of Cole and Favour (13), who found that both Boyden-type antibodies and those responsible for tuberculin hypersensitivity existed in the same fraction of guinea pig plasma.

Groups of rabbits were injected intravenously with either 2.0 mg. of BCG, or *M. phlei*, or 0.25 mg. of bovine tubercle bacilli. At the intervals recorded in table 3, serum samples were obtained from each of 2 animals prior to tuberculin testing with first-, intermediate-, and second-strength tuberculin (PPD). The tuberculin tests were read after forty-eight hours. All sera were concentrated and tested for agar-diffusing antibodies with OT. None of the animals was tested more than once.

Agar-diffusing antibodies were observed in some sera of animals injected with

2.0 mg. of BCG or *M. phlei*. For the most part, their presence paralleled the development of tuberculin hypersensitivity, although discrepancies were noted. The absence of agar-diffusing antibodies in the sera of rabbits infected with virulent bovine tubercle bacilli can be explained at present only on the basis of dosage (*see* Discussion).

DISCUSSION

By employing agar-diffusion techniques, it was possible to detect antiprotein substances in the sera of animals injected with various mycobacteria. The protein specificities of these antibodies were determined by their reaction with PPD and with OT from which the tuberculopolysaccharides had been adsorbed with normal sheep cells. Since more than one line of precipitation was frequently observed, it is probable that several antigen-antibody systems were being detected.

The most consistent demonstration of multiple precipitates occurred with an hyperimmune serum prepared against viable BCG, thus suggesting that at least some of the antibodies were produced against minor antigens in the bacillus. In order to demonstrate these antibodies in the sera of animals which had received a single injection of other mycobacteria, it was often necessary to concentrate the serum by desiccation despite the fact that 2.0 mg. of inocula were employed. Further evidence that stimulation of these antibodies required a relatively large concentration of antigen was afforded by the failure to demonstrate these substances in the sera of rabbits twenty-seven days after injection of 0.25 mg. of virulent tubercle bacilli.

By the antigens and methods utilized in this study to reveal the presence of antiproteins, it was possible to show that tanned cells adsorb different proteins from OT and from PPD. Also, it seems likely that the hemolytic demonstration sometimes observed with PPD-tanned cells is attributable to an antigen distinct from that responsible for hemagglutination. The inability to demonstrate hemolysis with any of the sera tested and OT-tanned cells is noteworthy and has been described previously (6).

Of particular interest is the apparent independence of at least some of the agar-diffusing antibodies from the antiproteins demonstrated with sensitized erythrocytes. Support for this was found in two observations: the absence of agar-diffusing substances in concentrated sera which when untreated reacted with protein-sensitized tanned cells; and the occasional demonstration of these antibodies in sera which had not reacted with the protein-treated erythrocytes.

The development of tuberculin hypersensitivity in infected animals without the concomitant demonstration of the agar-diffusing antibodies (table 3) was presumptive evidence that distinct specificities were involved. These animals each received an injection of 0.25 mg. of organisms which, although sufficient to induce infection and tuberculin reactivity, probably contained too little of the antigen responsible for the development of agar-diffusing antibody. Despite subsequent multiplication of the bacilli, a critical concentration of antigen was not attained during the test period. This explanation has some support from the

data presented in table 1 from which it may be recalled that the positive tests observed were with sera from rabbits injected with large inocula of virulent Ravenel organisms (2.0 mg.). However, further investigation of this aspect of the study is indicated.

SUMMARY

Agar diffusion antibodies possessing protein specificities were found in some sera of rabbits injected with mycobacteria. These substances are often present in low concentrations and may require concentration of the test serum for their demonstration.

Evidence has been presented which suggests that some of these antibodies may have specificities for proteins different from those observed with the sensitized-erythrocyte techniques employed. Presumably these antibodies are not associated with those responsible for tuberculin activity.

SUMARIO

Anticuerpos Tuberculosos Descubiertos por la Difusión en Agar: I. Especificidad e Incidencia en Sueros de Conejos de Anticuerpos Difundidos en Agar

En algunos sueros de conejos inyectados con micobacterias, se descubrieron anticuerpos difundidos en agar que poseían especificidad para las proteínas. Estas substancias se encuentran a menudo presentes en concentraciones bajas y tal vez requieran concentración del suero ensayado para descubrirlas.

Preséntanse datos que sugieren que algunos de esos anticuerpos pueden poseer especificidades para las proteínas distintas de las observadas con las técnicas de los eritrocitos sensibilizados empleadas. Presuntamente, esos anticuerpos no están asociados con los que intervienen en la actividad de la tuberculina.

RESUME

Anticorps tuberculeux démontrés par la diffusion dans la gélose: I. Spécificité et incidence des anticorps du sérum de lapin diffusant dans la gélose

Des anticorps diffusant dans la gélose et possédant les caractères spécifiques de la protéine ont été trouvés dans certains sérums de lapin inoculés avec les mycobactéries. Ces substances sont souvent présentes à faible concentration et peuvent réclamer la concentration du sérum testé pour les découvrir.

Les auteurs fournissent des données qui suggèrent que certains de ces anticorps peuvent exercer des actions spécifiques sur des protéines qui diffèrent de celles observées avec l'emploi des techniques utilisant l'érythrocyte sensibilisé. Il est probable que ces anticorps ne sont pas les mêmes que ceux responsables de l'activité de la tuberculose.

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TUBERCULOUS ANTIBODIES DEMONSTRATED BY AGAR DIFFUSION[1, 2]

II. Further Characterization of These Antibodies and Their Distribution in Human Tuberculous Sera

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INTRODUCTION

The demonstration of agar-diffusing antibodies with protein specificities in certain sera from rabbits injected with mycobacteria (1) suggested the testing of human tuberculous sera by the same technique. The results were compared with those from other serologic procedures as in the preceding paper. In addition, sera were fractionated to determine in which globulin fractions the various antibodies resided.

MATERIALS AND METHODS

The serologic techniques employed in the present series of experiments were similar to those described in the preceding paper, but with the following single modification.

To retard the nonspecific opacity that is commonly observed with some human sera in the agar-diffusion tests, 1 M glycine was introduced into the agar during its preparation (2). The pH was adjusted as before.

Sera: In addition to the antisera recovered from rabbits injected with mycobacteria, 40 human tuberculous sera, each from a different patient, and provided by the staff of the Ohio Tuberculosis Hospital, were included in this study. The kind and extent of antimicrobial therapy which the patients were undergoing at the time the sera were procured for the antibody tests consisted of isoniazid, streptomycin, PAS, and pyrizinamide in various two or more drug combinations for as short a time as two weeks and up to four years. Control sera were obtained from children three to nine years of age from Children's Hospital of Columbus.

Serum fractionation: Two procedures for serum fractionation were utilized in these experiments: the methanol technique as described by Pillemer (3) and the ethanol fractionation as reported by Deutsch (4). Minor changes in the techniques from those recommended by the authors necessitate their description.

I. *Methanol:* Ten volumes of serum were mixed with 7.5 volumes of deionized water and 5 volumes of acetate buffer (ionic strength 0.4, pH 5.4). The mixture was chilled to 1°C., after which 7.5 volumes of methyl alcohol (clinically pure), previously cooled to 1°C., were added at the rate of 0.25 volumes per minute. The precipitate which formed after one hour in an ice bath was removed following centrifugation at 2,500 r.p.m. for thirty minutes at 1°C. This precipitate was essentially pure beta globulin and was soluble in phosphate-buffered saline (pH 7.4).

II. *Ethanol:* One volume of serum was diluted with 3 volumes of deionized water. After lowering the temperature of the diluted serum to 0°C., 4 volumes of 50 per cent ethanol (precooled to −10° to −20°C.) were introduced slowly and with continuous stirring. The mixture then was chilled to −6°C. and held at this temperature for thirty minutes. After centrifugation at the same temperature (2,500 r.p.m., thirty minutes), the precipitate (*A*) was removed and the supernatant fluid was mixed with one volume

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[2] This investigation was supported, in part, by a grant from the Ohio Tuberculosis and Health Association.

of cold deionized water. The pH was adjusted to 5.1 with 0.05 M acetic acid, and the solution was placed in the ice bath for ten minutes, during which time precipitate *B* formed. The supernatant liquid was recovered after centrifugation at 0°C. and adjusted to pH 7.4 with 0.5 M sodium bicarbonate. An equal volume of 50 per cent ethanol was added slowly, and the temperature of the mixture was again lowered to −6°C. Under these conditions precipitate *C* appeared and was recovered in the manner described above.

All precipitates were reconstituted to the original serum volume with isotonic saline before being tested for antibody content. It was found necessary to add dry sodium chloride to the supernatant liquids to achieve isotonicity.

Electrophoresis: Electrophoretic mobilities of the various serum fractions were determined by paper electrophoresis in a Durram tent-type tank and a variable power source (Heathkit, Model PS-3). Adequate separation of fractions was obtained with 3 MM filter paper and veronal buffer (pH 8.6, ionic strength 0.05) after five hours at a current of 25 milliamperes. The paper strips were dried in a hot-air oven (170°C.) for ten to fifteen minutes and stained for four hours by immersion in a 0.1 per cent solution of bromphenol blue. After several ten-minute washings in 2.0 per cent acetic acid and subsequent development in sodium acetate solution (0.75 per cent), the strips were re-dried and examined.

RESULTS

Distribution of Antibodies in Human Sera

Forty human tuberculous sera and an equal number of control sera (from children, three to nine years of age and presumably tuberculin negative) were concentrated and tested for reactivity in agar with various dilutions of OT. In addition, each serum sample was examined for antituberculopolysaccharides and antiproteins by the sensitized-erythrocyte techniques (*see* previous paper for description of antibody demonstrations (1)).

In no instance were reactions noted with the control sera. The distribution of reactions among the tuberculous sera is recorded in table 1, in which it may be seen that only 5 of the 40 sera tested (12.5 per cent) demonstrated agar-diffusing antibodies. It should be mentioned that fewer lines of precipitation were observed with the human sera than were seen with the hyperimmune anti-BCG sera from rabbits. These same human sera contained antiproteins as determined by their reactivity with protein-sensitized erythrocytes. However, 15 other sera,

TABLE 1

DISTRIBUTION OF ANTIBODIES IN TUBERCULOUS SERA

Number of Sera	Agar-Diffusing Antibodies	Antituberculopoly-saccharides	Antituberculoproteins
0	+	−	−
0	+	+	−
5	+	+	+
0	+	−	+
14	−	+	+
13	−	+	−
1	−	−	+
7	−	−	−
Total 40	5 (12.5%)	32 (80%)	20 (50%)

which also reacted with sensitized erythrocytes, failed to exhibit antibodies by the agar-diffusion test. Thirty-two of the sera (80 per cent) gave positive reactions for antituberculopolysaccharides.

Antibody Content of Serum Fractions

In order to further characterize the agar-diffusing antibodies, both positive human sera and sera from rabbits hyperimmunized with BCG were fractionated by the methanol procedure. The fractions tested for serologic activity proved to be essentially beta globulin as shown by paper electrophoresis. Although occasional low titers of antipolysaccharides were observed in this fraction from some rabbit sera, none of the fractions tested reacted with OT in the agar procedure.

Having established the absence of these antibodies in the beta globulin fraction, two anti-BCG sera (rabbits) were fractionated by the method of Deutsch (4). The four fractions obtained were subjected to electrophoresis (figure 1) and serologic examination. The results of testing one such fractionation are recorded in table 2, from which it may be seen that the agar-diffusion antibodies

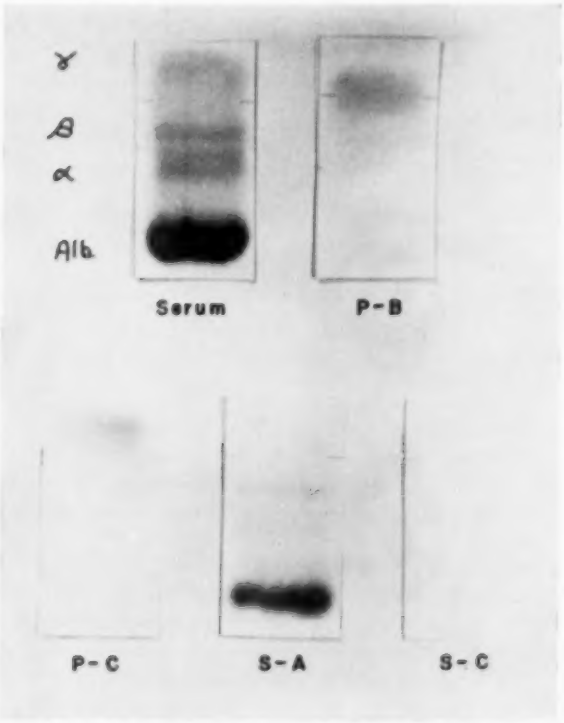

FIG. 1. Electrophoretic separation of serum fractions

TABLE 2

ANTIBODY CONTENT OF SERUM FRACTIONS

Fraction	Components	Antipolysaccharide		Antiprotein	
		HA*	HL*	HA	HL
Supernate *A*	Mostly albumin, but also small amounts of α, β, γ_1, and γ_2 globulins	—	64†	—	—
Supernate *C*	Trace quantities only	—	—	—	—
Precipitate *B*	β and small amounts of γ_2	—	4	—	—
Precipitate *C*	γ_2 globulin	16	128	—	—
Whole serum		128	512	8	—

* H.A. refers to hemagglutination, H.L. refers to hemolysis.

† All titers expressed as reciprocals of serum dilution.

TABLE 3

FRACTIONATION OF HUMAN SERA

Serum Number	Agar Reactivity		Antipolysaccharide				Antiprotein			
	Whole Serum	Serum Fractions	Whole Serum		Serum Fractions		Whole Serum		Serum Fractions	
			HA*	HL*	HA	HL	HA	HL	HA	HL
B-7	+	+ (Precipitate *C*)	16	64	—	32 (Precipitate *B*)	8	—	8	— (Precipitate *C*)
					2	32 (Precipitate *C*)				
C-4	—	—	8	8	4	2 (Precipitate *C*)				
C-1	—	+ (Precipitate *C*)	—	—	—	—	4	—	—	—
D-15	—	—	8	4	—	—	—	—	—	—

* H.A. refers to hemagglutination; H.L. refers to hemolysis.

and the preponderence of antipolysaccharides were contained in the gamma globulin-bearing fractions. Although the Boyden antibodies (antiproteins, 5) were not demonstrated in any of the fractions of the example shown in the table, similar tests with other fractionated hyperimmune sera indicated their presence in the gamma globulins.

For purposes of comparison, 4 tuberculous human sera were fractionated and tested in a like manner. These sera were selected because of their variation in serologic reactivity. As shown in table 3, the antiproteins, including the agar-diffusing substances, and the antipolysaccharides were found in the fractions containing gamma globulins, which is in agreement with the results observed from testing the rabbit sera. Of interest was the demonstration of agar-diffusing antibodies which occurred only after fractionation of serum C-1.

DISCUSSION

The incidence of agar-diffusing antibodies in human tuberculous sera is considerably less than was seen in the sera from rabbits which had received large inocula of mycobacteria intravenously, as reported in the preceding paper. If the assumption is valid that a relatively large concentration of antigen(s) is necessary for stimulating the production of these substances, a testing procedure such as this might warrant further investigation as a possible aid in prognosis. Although the number of sera tested admittedly was small, the markedly fewer reactions observed by this technique as compared with those demonstrated with sensitized erythrocytes are more in keeping with the ameliorative effects generally observed with modern therapy.

Again, as was concluded from the previous study with rabbits, the agar-diffusion antibodies observed in human sera probably are distinct from those capable of inducing tuberculin sensitivity since it is highly unlikely that only 5 of the 40 tuberculous patients from whom sera were procured would be tuberculin reactors. No attempt was made to relate the specificities of the agar-diffusing antibodies in human sera with those observed in the rabbit sera other than to ascertain that in both instances the antibodies were directed toward proteins or protein complexes.

With regard to the distribution of the various antibodies in serum fractions, it would appear, at least from the present experience, that the antipolysaccharides, agar-diffusing antiproteins, and the Boyden-type antibodies all were associated with gamma globulin-bearing fractions although their presence was not mutually dependent. This was true of both rabbit and human sera.

Cole and Favour (6) have reported that, while the antipolysaccharides (Middlebrook-Dubos antibodies) reside in the gamma globulin fraction of hyperimmunized guinea pigs, the antiproteins (Boyden antibodies) were found in a new fraction designated as IV-10 which also was capable of passively transferring tuberculin hypersensitivity. The residence of the antiproteins in rabbit and human sera as compared with the results just cited with guinea pig sera can best be explained on the basis of species differences, although the intensity of the immunization in the test animals must also be considered.

Little can be said concerning the demonstration of only agar-diffusing antibodies after the fractionation of the human serum, C-1 (table 3). Gerstl and his associates (7) have shown that re-examination of certain sero-negative tuberculous human sera following fractionation resulted in positive tests for antipolysaccharides. The possible presence of inhibitor was suggested as being responsible for the false-negative reactions. Whether or not an analogous phenomenon could account for the results presented here is as yet undetermined.

SUMMARY

Five of 40 tuberculous human sera contained agar-diffusing antibodies with specificities directed to antigens present in Old Tuberculin. An equal number of sera from nontuberculous children failed to react.

The antipolysaccharides, agar-diffusing antibodies, and the antiproteins demonstrated with protein-sensitized erythrocytes were found to be associated

with the gamma globulin-bearing fractions in both the human test sera and the sera from rabbits injected with BCG.

SUMARIO

Anticuerpos Tuberculosos Descubiertos por la Difusión en Agar: II. Mayor Caracterización de Estos Anticuerpos y Su Distribución en Sueros Tuberculosos Humanos

Cinco de 40 sueros humanos tuberculosos contenían anticuerpos que se difundían en agar y poseían especificidades relacionadas con antígenos presentes en la tuberculina de Koch. Un número igual de sueros procedentes de niños no tuberculosos no reaccionó.

Obsérvóse que los antipolisacáridos, los anticuerpos que se difundían en agar y las antiproteínas descubiertas con eritrocitos proteíno-sensibilizados se asociaban con fracciones que contenían la globulina gamma tanto en los sueros humanos ensayados como en los sueros de conejos inyectados con BCG.

RESUME

Anticorps tuberculeux démontrés par la diffusion dans la gélose: II Autres caractéristiques de ces anticorps et leur répartition dans le sérum humain tuberculeux

Parmi 40 sérums humains, 5 renfermaient des anticorps diffusant dans la gélose. Ces anticorps exercaient une action spécifique contre les antigènes présents dans la vieille tuberculine. Des sérums, en nombre égal, prélevés chez des enfants n'ont pas présenté cette réaction.

Les antipolysaccharides, les anticorps diffusant dans la gélose et les antiprotéines identifiés à l'aide d'érythrocytes sensibilisés à la protéine ont été trouvés associés aux fractions globuline gamma, tant dans les sérums humains testés que dans les sérums de lapins inoculés avec le B.C.G.

Acknowledgment

The writers wish to express their sincere appreciation to Dr. Joseph Tomashefski, Mrs. Robert MacMillan, and Mr. Samuel Fraley of the Ohio Tuberculosis Hospital, Columbus, Ohio, for their advice and assistance throughout the course of this study.

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VACCINATION OF MICE AGAINST COCCIDIOIDES IMMITIS[1,2,3]

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(Received for publication March 20, 1956)

INTRODUCTION

A large percentage of the population of the arid regions of the Southwest become infected with the fungus, *Coccidioides immitis*. Approximately 40 per cent of the infections are symptomatic. The majority of these are of a mild to acute "influenzal" or "pneumonic" type which is self-limited, but occasionally the infection becomes extrapulmonary and then is often fatal. There is no specific chemotherapy. However, epidemiologic evidence (1) as well as experimental studies indicate that a single infection confers a relatively solid immunity.

Although the need for prophylaxis is great, efforts to immunize against this fungus have met with little success. Recently Vogel and associates (2) attempted to immunize guinea pigs with heat-killed spherules of *C. immitis*. The animals developed sensitivity to coccidioidin, but a comparison of the lesions in the vaccinated animals and untreated controls showed only a relative increase in resistance. It may well be that their use of guinea pigs diminished the clarity of results, for more than ten years ago one of the present writers (C.E.S.), attempting vaccination of this animal with heat-killed coccidioides, failed to demonstrate conclusive protective effect. The present report describes the protection of mice by vaccination with killed spores of the saprophytic phase of *C. immitis*.

MATERIAL AND METHODS

Strains[5] of *C. immitis* were cultured as described in previous studies as yet unpublished (3).

Vaccines of *C. immitis* were prepared by two methods: (*A*) submerged culture of the Cash strain was centrifuged and the cells were re-suspended in acetone. This suspension was macerated at −70°C. in a tissue grinder. The acetone was almost completely removed by evaporation and the sediment was re-suspended to a concentration of 19 per cent by volume in saline solution. This percentage by volume, as well as those to be mentioned subsequently, was determined by centrifugation in a Wintrobe hematocrit tube. (*B*) Two lots of dry spores from agar cultures of the Silveira strain were killed with formaldehyde by

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[2] These investigations were conducted in part under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board; in part by the Office of the Surgeon General, Department of the Army; and in part by a contract between the University of California and the Office of Naval Research.

[3] The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (article 1252, U. S. Navy Regulations, 1948).

[4] Present address: Department of Microbiology, School of Medicine, Tulane University, New Orleans, Louisiana.

[5] Obtained from the collection maintained at the School of Public Health, University of California, in connection with research supported by the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board.

placing them above concentrated formalin in a closed container at 34°C. They were then left exposed at room temperature until free of formaldehyde odor. Lot No. 1 was suspended to a concentration of 21 per cent by volume in saline solution; the other lot, to 27 per cent.

In addition to the specific agent, saline suspensions of wood flour, rice starch, and viable, broth-grown *Saccharomyces cerevisiae* were prepared. The concentrations were adjusted to 20 per cent by volume. The nonantigenic wood and starch were injected to rule out nonspecific protection which might be produced by injection of an inert particulate substance. *S. cerevisiae* was included to observe possible cross immunization by another fungus.

In groups of 28 to 39, white mice were inoculated subcutaneously with 0.1 ml. of the above suspensions at the ninth, thirteenth, fifteenth, seventeenth, and twenty-second weeks prior to challenge. These inoculated mice and 100 normal mice were challenged by the intraperitoneal route with one hundred viable spores of the Silveira strain. The mice were observed for sixty days and at the end of this period all survivors were sacrificed and autopsied for evidence of infection. Cultures on 3 per cent malt agar were made of lesions and of lungs and liver when no lesions were observed.

RESULTS

Protection was conferred by each of the three vaccines prepared from *C. immitis*. As shown in figure 1, vaccination markedly reduced the lethality. There

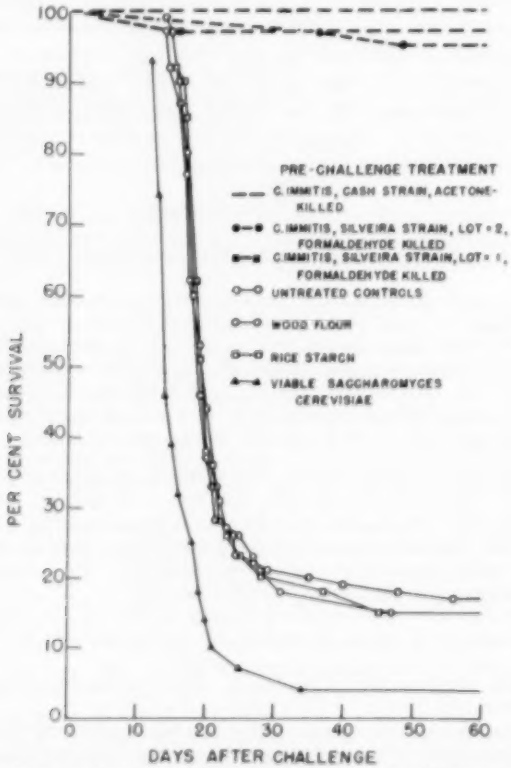
PER CENT SURVIVAL
DAYS AFTER CHALLENGE
PRE-CHALLENGE TREATMENT
C. IMMITIS, CASH STRAIN, ACETONE-KILLED
C. IMMITIS, SILVEIRA STRAIN, LOT • 2, FORMALDEHYDE KILLED
C. IMMITIS, SILVEIRA STRAIN, LOT • 1, FORMALDEHYDE KILLED
UNTREATED CONTROLS
WOOD FLOUR
RICE STARCH
VIABLE SACCHAROMYCES CEREVISIAE

FIG. 1. The survival of mice vaccinated against *Coccidioides immitis*

was only one death in the animals which received lot No. 2 of the formaldehyde-killed spores of the Silveira strain, and 2 in those which received lot No. 1 of the same strain. There were no deaths among the animals which were inoculated with the acetone-killed vaccine of the Cash strain. By comparison (4), half of the untreated control animals were dead in nineteen days and 80 per cent were dead at the end of the observation period of sixty days.

Although the course of the disease was favorably modified in the vaccinated animals, the infectivity of *C. immitis* was only slightly reduced. At autopsy, sixty days after challenge, only 3 (10 per cent) of the survivors which received the acetone-killed Cash vaccine and only 4 (13 per cent) of those inoculated with lot No. 2 of the Silveira strain appeared normal and gave negative cultures. However, 15 (38 per cent) of the mice which had been vaccinated with formaldehyde-killed Silveira, lot No. 1, had neither gross lesions nor positive cultures. The vaccine prepared from lot No. 2 and lot No. 1 of the Silveira strain differed in the concentration of cells (27 and 21 per cent, respectively). It is unlikely that the difference in results is attributable to this factor since concentration of the vaccine would perhaps favor lot No. 2 which afforded less protection to infection. All of the control animals, including those which received wood flour, starch, and the yeast, were infected. Similarly, Salvin (5) found that the tissue of mice immunized against *Histoplasma capsulatum* was infected but not to the same extent as nonimmunized control mice.

The similarity of the survival curves (figure 1) of the control mice and those which received the wood and the starch indicates that no protection was afforded by those inert particulate substances. *S. cerevisiae* actually seemed to lower resistance to challenge with *C. immitis*. This phenomenon has been studied by Pappagianis (6) who suggested the use of this yeast in the present study. Scherr (7) also reported the lowering of host resistance to a fungus, *Candida albicans*, by *S. cerevisiae*.

DISCUSSION

The factors responsible for the protection observed in the present studies with killed *C. immitis* were not clear. Apparently the strain differences were not critical since the Cash strain evoked as good protection against lethality by the Silveira strain as the vaccine prepared from the homologous Silveira strain. Moreover, the efficacy was similar whether formaldehyde or acetone was used to kill the organism or whether the fungus used for the vaccine had been grown in submerged culture in liquid medium or on the surface of a solid medium.

Since an immune response was readily induced under a variety of conditions, the absence of reports of successful immunizations may be related to the experimental host employed. Both Negroni and associates (8) and Vogel and co-workers (2) used guinea pigs. In this animal the development of coccidioidomycosis is extremely variable and the results are often difficult to interpret. In the experience of the present writers, the infection pattern in mice is much more homogeneous and reproducible.

Many obvious questions have not been answered. For example, is the response to the vaccine sufficient to protect against a greater challenge? How durable is

the immunity? Although the severity of the infection was less in the vaccinated animals than in the controls, the high proportion of infection demonstrable by autopsy at the end of sixty days makes one very cautious. Might these animals have succumbed if the period of observation had been extended? The practicality of human vaccination cannot be estimated from these preliminary experiments, but the immune response in the animals used in these experiments is justification for continued efforts to develop an appropriate vaccine.

SUMMARY

Mice which were vaccinated subcutaneously with killed spores of *Coccidioides immitis* showed an increased resistance to challenge by viable *Coccidioides immitis*. This resistance was evidenced by survival for sixty days of 95 to 100 per cent of the vaccinated mice compared with 20 per cent of the control animals. However, autopsy revealed that nearly all survivors were infected with the fungus.

SUMARIO

Vacunación de los Ratones contra el Coccidioides Immitis

Los ratones vacunados intraperitonealmente con esporos matados del *Coccidioides immitis* revelaron aumento de su resistencia a la provocación con *Coccidioides immitis* viables. Esta resistencia quedó demostrada por la sobrevivencia durante sesenta días de 95 a 100 por ciento de los ratones vacunados, comparado con 20 por ciento de los testigos. No obstante, la autopsia reveló que casi todos los sobrevivientes estaban infectados.

RESUME

Vaccination des souris contre le Coccidioides immitis

Les souris vaccinées par voie intrapéritonéale avec des spores tuées de *Coccidioides immitis* présentaient une résistance accrue à l'inoculation par les *Coccidioides immitis* viables. Cette résistance était démontrée par la survie de l'animal pendant soixante jours, chez 95% des souris vaccinées par rapport aux 20% enregistrés chez les souris témoins. Cependant, l'autopsie a révélé que presque toutes souris survivantes étaient infectées.

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A MODIFICATION OF VIRULENCE OF MYCOBACTERIUM TUBERCULOSIS BY CHICK EMBRYO ADAPTATION[1, 2]

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INTRODUCTION

It is well known that birds are highly resistant to infection with the human tubercle bacillus. In 1948, Eggerth, Dresher, and McOsker (1) found that there was a progressive increase in virulence for chick embryos of a human type *N* strain of *Mycobacterium tuberculosis* after nine to fifteen passages through embryonated eggs. Since this was done only with a single strain, further confirmation is advisable. It also may be thought to be of interest to note whether or not tubercle bacilli, following passage through embryonated eggs, will develop an altered virulence for the guinea pig. A controlled modification of the virulence of strains of *M. tuberculosis* might have potential value for practical applications.

In view of the above interest, four strains of *Mycobacterium tuberculosis* freshly isolated in this laboratory were chosen for this study. The purpose of the present report is to present the results of the effects on virulence of the tubercle bacillus in chick embryos and experimental animals by serial propagation in embryonated eggs.

MATERIALS AND METHODS

Organisms: Four strains of tubercle bacilli were inoculated into rabbits and guinea pigs; three were identified as *M. tuberculosis* var. *hominis* and one was classified as *M. tuberculosis* var. *bovis*.

M. tuberculosis var. *hominis* strains:

Addison strain was isolated from a patient in the Grace-New Haven Community Hospital. Primary isolation of this organism was from the yolk of embryonated eggs inoculated with digested sputum (2). The organism was susceptible to streptomycin (1 mg. per ml.) and isoniazid (0.06 mg. per ml.).

Smith strain was isolated from a patient in the Laurel Heights Sanatorium, Shelton, Connecticut. Tubercle bacilli were found abundantly in the yolk of embryonated eggs during primary isolation. This organism was resistant to streptomycin (30 mg. per ml.) and susceptible to isoniazid (0.3 mg. per ml.).

Fletcher strain was isolated from a patient in Laurel Heights Sanatorium. Saline suspension of the growth on Löwenstein-Jensen slant was used for the first passage into yolk sacs of embryonated eggs. The organism was resistant to both streptomycin (30 mg. per ml.) and isoniazid (1 mg. per ml.).

M. tuberculosis var. *bovis* strains:

Crissey strain killed the rabbit twenty days after subcutaneous inoculation of a broth culture. Primary isolation of this strain was on penicillin blood agar (3). A saline suspension of a two-week-old culture was inoculated into yolk sacs of em-

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[2] Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

bryonated eggs. This organism was susceptible to streptomycin (1 mg. per ml.) and resistant to isoniazid (1 mg. per ml.).

Procedure for Serial Propagation of Tubercle Bacilli in Embryonated Eggs

Six- to seven-day-old embryonated eggs were used for all the passages. Methods of candling the eggs and inoculation were those of Beveridge and Burnett (4). Saline suspensions of the strains of *M. tuberculosis* mentioned above were used as inoculum. Two-tenths ml. of a suspension were inoculated into each of six yolk sacs; the perforations in the egg shell were sealed with scotch tape and the eggs were incubated at 37°C. Four days after inoculation, the eggs were candled and the dead embryos discarded. On the twentieth day of incubation the surviving eggs were chilled for two hours to kill the embryos. The eggs were then opened aseptically. Approximately 1 gm. of the yolk content was collected into a sterile test tube and 2 ml. of sterile saline were added to make a uniform suspension. Each subsequent experiment involved the inoculation of yolk from the preceding run into six embryonated eggs as described above. Six eggs were used each time to allow for loss through traumatic death of some of the embryos.

Before each series of inoculations was begun, acid-fast smears were made to check for presence of organisms. Infected embryos were removed and examined for gross lesions of the internal organs. Spleen and liver were saved for histologic sections in every fifth or sixth passage. These tissues were fixed in 10 per cent formalin, imbedded in paraffin, and sections were stained by a modified Ziehl-Neelsen method (5).

Four strains of egg-adapted *M. tuberculosis* were obtained for the virulence test in guinea pigs:

Addison XXV: Addison strain, twenty-five passages in embryonated eggs
Smith XXIV: Smith strain, twenty-four passages in embryonated eggs
Fletcher XV: Fletcher strain, fifteen passages in embryonated eggs
Crissey XVIII: Crissey strain, eighteen passages in embryonated eggs

Preparation of Yolk Suspensions and Broth Cultures for Animal Inoculation

Yolk suspensions: Yolk infected with egg-adapted strains of tubercle bacilli was harvested thirteen to fourteen days after inoculation and suspended in sterile saline in a dilution of 1:2. The mixture was shaken vigorously for ten minutes and centrifuged at low speed for ten minutes in order to remove larger bacillary clumps. The supernatant was collected and a tenfold serial dilution was made in sterile saline. Colony counts were made by inoculating 0.01 ml. of each dilution onto a portion of penicillin blood agar plate, using a tuberculin syringe and beginning with the highest dilution. Three plates were used for each determination and they were incubated at 37°C. The remaining undiluted yolk suspension was frozen immediately at −20°C.

After two weeks of incubation, colonies on the blood agar plates were counted, and the average number of viable tubercle bacilli (or bacillary particles) per milliliter was evaluated. The frozen yolk suspension was then thawed and a desirable dilution of a known amount of viable bacilli (as enumerated from the colony counts) was used for animal inoculation. In the meantime, plate counts of the tenfold dilutions of the frozen yolk suspension were repeated to check for possible loss of viable cells during the period of storage.

Broth cultures: Pure cultures of the four unadapted strains of tubercle bacilli, grown on blood agar slants, were inoculated separately into Bacto-Dubos broth with 0.02 per cent Tween® 80 and 10 per cent Dubos medium and serum. After twelve to fourteen days of incubation at 37°C., the broth cultures were shaken vigorously and centrifuged. Plate-counting technique was done as far as the yolk suspension in order to determine the number of viable bacilli per milliliter.

Virulence Test of the Egg-Adapted Strains of Tubercle Bacilli in Embryonated Eggs and in Guinea Pigs

Embryonated eggs: All of the embryonated eggs used in the experiment were six to seven days old and the route of inoculation was the yolk sac. In each test six eggs received yolk

suspension of an egg adapted strain of tubercle bacilli containing approximately 200,000 viable cells, while another six eggs received an equal amount of organisms of the respective unadapted strain grown in broth culture for twelve to fourteen days. All of the inoculated eggs were incubated at 37°C. At the end of two weeks' incubation, the eggs were opened and the embryos were examined for pathologic changes.

Guinea pigs: Male guinea pigs weighing approximately 250 to 400 gm. were used in this experiment. These animals were negative when skin-tested with 0.1 ml. of 0.005 mg. of tuberculin (PPD second-strength). Inoculations of the yolk suspension and the broth cultures into guinea pigs were made subcutaneously in the inguinal region with a known amount of viable tubercle bacilli. In every experiment each of 3 guinea pigs received the yolk suspension of an egg-adapted strain of tubercle bacilli containing approximately 2,000 viable cells, while 3 others received equal amounts of organisms in a broth culture of the respective unadapted strain. These two groups of animals were kept in two separate cages but housed in the same animal room. At the end of four, six, and eight weeks the animals were sacrificed, one from each group. Spleens were used as an index of the degree of infection. Liver and lung were examined for gross lesions, and histologic sections were made in the questionable cases. Cultures were made from the enlarged regional lymph nodes and spleen on blood agar plates.

RESULTS

Observations on Serial Propagation of M. Tuberculosis in Embryonated Eggs

During the first five passages of the Addison strain there was no gross evidence of effect on virulence to chick embryos although scattered tubercles were present over the chorio-allantoic membranes along the blood vessels. The thickness of the membranes was not increased. Smears of the yolk did show increased numbers of tubercle bacilli, and histologic sections revealed a few acid-fast organisms in the spleen and the liver.

Following the tenth to twelfth passages, the infected embryos appeared smaller in size than did normal embryos. The surfaces of the moderately enlarged spleens were studded with a few tubercles while the chorio-allantoic membranes were covered with numerous pinpoint tubercles or clusters of tubercles.

After fifteen passages the embryos (figure 1) were greatly stunted, curled, and possessed spleens markedly enlarged with numerous tubercles. The chorio-allantoic membranes were thickened with confluent plaques and tubercles. Embryos inoculated with the yolk suspension of this organism following the twentieth passage did not reveal any significant changes in virulence beyond those just noted and were still viable.

Results similar to those described for the Addison strain were also obtained for the Smith, Fletcher, and Crissey strains. It was noted that all membranes of viable embryos following inoculation with yolk suspensions of the Crissey strain after the eleventh passage were markedly thickened and had many confluent plaques (figure 2).

Investigations on the Virulence of Egg-Adapted Tubercle Bacilli in Guinea Pigs

A total of 41 guinea pigs were inoculated with the different egg-adapted strains and the unadapted strains. The results obtained from each animal are indicated in figure 3.

Guinea pigs inoculated with the Addison strain following twelve passages through chick embryos showed marked weight loss and emaciation, followed by

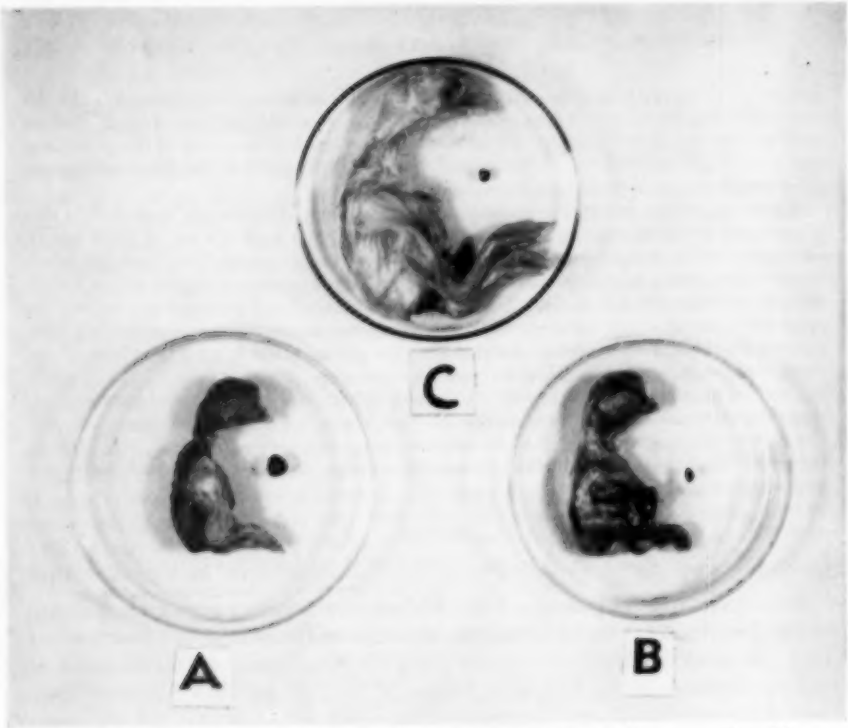

FIG. 1. Gross appearance of the freshly removed spleens and the twenty-day-old embryos: *A*. Infected with egg-adapted Addison XXV in yolk suspension; *B*. Infected with the unadapted Addison strain in broth culture; *C*. A normal embryo.

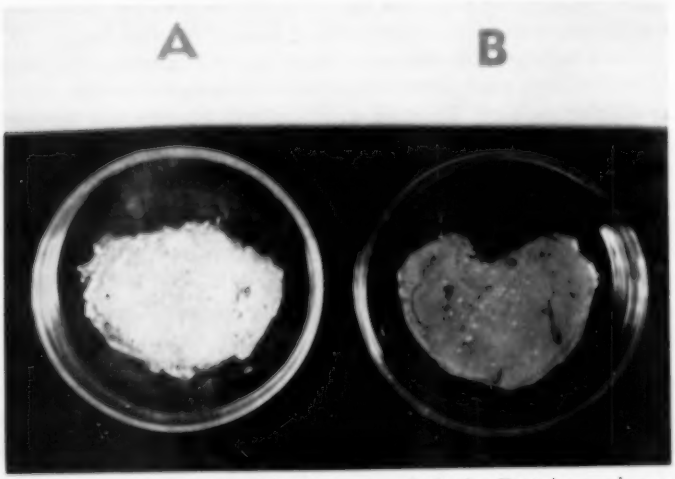

FIG. 2. Gross appearance of the freshly removed chorio-allantoic membranes of the twenty-day-old chick embryos infected with equal numbers of

A. Egg adapted Crissey XVIII in yolk suspension

B. Unadapted Crissey strain in broth culture

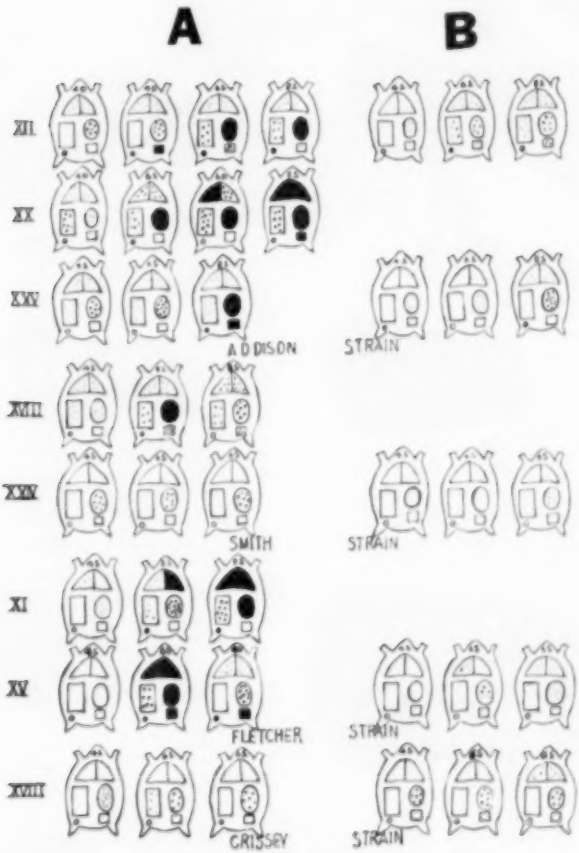

FIG. 3. Extent of tuberculosis in guinea pigs at necropsy after infections with (*A*) egg adapted and (*B*) unadapted strains of tubercle bacilli.

spleen
mesentery lymph nodes
liver
site of inoculation and regional lymph node
D number of weeks after injection animal died
S number of weeks after injection animal sacrificed

The extent of tuberculosis in the organs is indicated by the degree of shading:

maximal involvement
moderate involvement
minimal involvement
grossly no change

death of 2 animals at the end of the fourth week. This was in contrast to those animals which had been inoculated with the unadapted strain and were symptomless. All surviving animals were sacrificed at the end of six and eight weeks. Maximal tuberculous lesions were obtained in the pigs inoculated with the egg-

FIG. 4. Gross appearance of the formalin-fixed spleen of guinea pigs infected with equal numbers of (*A*) egg adapted Addison XXV in yolk suspension; (*B*) unadapted Addison strain in broth culture.

Top: Spleens from animals four weeks after infection
Center: Spleens from animals six weeks after infection
Bottom: Spleens from animals eight weeks after infection
Compare with the results in chick embryos simultaneously infected (figure 1)

adapted organisms, and minimal lesions were found in animals inoculated with the unadapted strain (figure 4).

The guinea pigs inoculated with egg-adapted strains of Smith and Fletcher also showed marked increase in virulence as contrasted to those animals inoculated with the unadapted strains (figures 3, 5).

FIG. 5. Gross appearance of the formalin-fixed spleens, mesentery lymph nodes, and lungs of guinea pigs infected (six weeks) with equal numbers of (*A*) egg adapted Fletcher XV in yolk suspension; (*B*) unadapted Fletcher strain in broth culture.

Note the comparatively small spleen and rare, small lesions on lung of animal in (*B*) group.

Guinea pigs inoculated with the Crissey strain adapted through chick embryos failed to show any increase in virulence (*see* figure 2) which is in marked contrast to the other strains.

DISCUSSION

Subcultures of yolk to new yolk sacs were made on the fifth, seventh, tenth, thirteenth, and fourteenth days following primary inoculation of chick embryos. Acid-fast stains failed to reveal any organisms in yolk subcultured on the fifth through the tenth days; a few organisms were seen in the yolks subcultured on the tenth day but, by the thirteenth or fourteenth day, abundant organisms were seen.

Moore (6), Emmart and Smith (7), and Fite and Olson (8) have used the chorio-allantoic membrane of chick embryos to study the virulence of tubercle

bacilli. Lee and Stavitsky (9) reported that intravenous inoculation of chick embryos with a strain of *M. tuberculosis* var. *hominis* resulted in a uniform infection of the parenchymatous organs. In this study it was observed that, when tubercle bacilli were introduced into the yolk sac of six- to seven-day old embryonated eggs, a uniform infection was obtained thirteen to fourteen days later. This method, compared to the above mentioned routes, was simpler and easier. Uniform suspension of the yolk material was easily obtained without further grinding. The yolk sac route, therefore, was chosen for all of the subcultures. Tubercle bacilli multiplied successfully in this way and serial passages were made in the yolk of six- to seven-day-old chick embryos.

The spleen of the embryo was always involved and offered gross evidence of infection. Thus, the appearance and size of the spleens usually served as an index of infection. It was not unusual to find gross lesions on the surface of the liver. Bacilli and lesions in the unexpanded lungs of embryos were unsatisfactory for the determination of the degree of infection.

The Crissey strain, which was classified as a bovine variant, showed enormous thickened tuberculous areas on the chorio-allantoic membrane after eleven passages. This organism, following its eighteenth subculture through embryonated eggs, gave no evidence of enhanced virulence in guinea pigs similar to those seen with the other three strains.

Emmart and Smith (7) and Moore (6) found that the virulence of tubercle bacilli could be determined as successfully in chick embryos as in guinea pigs. A virulent strain for guinea pigs produced many lesions of the chorio-allantoic membrane of chick embryos, whereas an avirulent strain produced little or no lesions. The present studies indicate that, with the exception of the Crissey strain, as the virulence of the organisms increased for the chick embryos, it also increased for the guinea pig.

The antigenicity of these strains of tubercle bacilli whose virulence has been enhanced by passage through embryonated eggs has not been studied. Whether these strains possess a better antigen for the hemagglutination test for tuberculosis or for the production of tuberculin remains to be determined.

SUMMARY

Three strains of *M. tuberculosis* var. *hominis* and one strain of *M. tuberculosis* var. *bovis* showed marked increase in virulence for chick embryos after ten to fifteen passages through embryonated eggs. With the exception of the bovine strain, a marked increased virulence of these strains was noted for the guinea pig.

The penicillin blood agar plate-counting method was used to determine the number of viable bacilli inoculated at all times. Reproducible colony counts were consistently obtained by this method.

SUMARIO

Modificación de la Virulencia del Mycobacterium Tuberculosis por Adaptación al Embrión de Pollo

Tres cepas del *M. tuberculosis* var. *hominis* y una cepa var. *bovis* revelaron notable aumento de su virulencia para los embriones de pollo después de diez a quince pases por

huevos embrionados. Exceptuada la cepa bovina, notóse también notable aumento de la virulencia de esas cepas para el cobayo.

Usóse el método de la numeración en placas de agar-sangre-penicilina para determinar el numero de bacilos viables inoculados en cada ocasión. Con este método se obtuvieron constantemente fórmulas reproducibles de las colonias presentes.

RESUME

Modification de la virulence du Mycobacterium tuberculosis par l'adaptation dans l'oeuf fécondé

Trois souches de *M. tuberculosis* var. *hominis* et une souche de *M. tuberculosis* var. *bovis* ont montré un accroissement marqué de leur virulence pour l'embryon de poulet après 10 à 15 passages dans l'oeuf fécondé. A l'exception de la souche bovine, un accroissement marqué de l virulence de ces souches pour le cobaye a été observé. La méthode de numération sur plaques pénicilline-sang-gélose a été utilisée pour dénombrer les bacilles viables inoculés à tout moment. Cette méthode a permis de reproduire constamment les numérations des colonies.

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ON THE PROBLEMS OF REINFECTION OF TUBERCULOSIS[1, 2]

The Use of Streptomycin-Resistant Tubercle Bacilli as Labeled Cells for Reinoculation Experiments

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With the editorial assistance of Gerald E. Muehsam[3]

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INTRODUCTION

The problem of reinoculated tubercle bacilli presents an important study in relation to human reinfection tuberculosis. In the Koch phenomenon (1) the site reinoculated with tubercle bacilli necrotizes in a few days, and the wound then localizes and heals. Large numbers of bacilli are lost with the necrotic tissue, but nothing is known about the tubercle bacilli which remain.

Pottenger (2), after reviewing the literature (3–7), concluded that "an inflammatory process surrounds the bacilli, immediately destroying and detaining others, then preventing them from spreading." This reasoning, however, is arrived at by analogy with other immunologic facts, and no experimental evidence exists tracing the fate of reinoculated tubercle bacilli in animal bodies.[4] This problem has until recently presented serious experimental difficulties. Reports are available of coexisting infection due to human and bovine types of tubercle bacill (8). Other investigators have attempted to follow the fate of reinoculated tubercle bacilli by using different strains of organisms (9–12). However, none of the methods seems to have given satisfactory results.

The present study represents an attempt to observe whether streptomycin-resistant tubercle bacilli (SM*R*TB) may be used for this purpose. These organisms are "tagged" by their reaction to streptomycin, and their fate can be followed. Since Youmans and Karlson and others (13–15) first demonstrated streptomycin-resistant tubercle bacilli, it has been shown that the streptomycin resistance of these organisms is permanent for many years both on culture media as well as in animal hosts.

The purpose of the study is to determine (*1*) whether preliminary inoculation can provide adequate protection against reinfection; (*2*) what, if this protection

[1] From the Central Laboratory of Fukushima Medical College, Fukushima, Japan.

[2] The outline of this paper was reported at the 1951–1953 annual meetings of the Japanese Association for Tuberculosis (22).

[3] The original manuscript submitted to the REVIEW by Dr. Osato and his associates was extremely detailed, and it was necessary to condense and revise it to conform to REVIEW style. Accordingly, at the request of Dr. Osato and his associates, the original manuscript was reorganized and condensed by Dr. Gerald E. Muehsam of the Veterans Administration Hospital, East Orange, New Jersey. Both the authors of the manuscript and the Editor of the REVIEW wish to express their appreciation to Dr. Muehsam for his very considerable contribution to the publication of this report.—*The Editor.*

[4] This problem has now been studied with the use of isotopically labeled BCG by Ström and by Rist (*see* Supplement to August, 1956, REVIEW).—*The Editor.*

can be provided, is the effect of the time interval between primary and secondary inoculation; and (*3*) the amount of reinoculated tubercle bacilli needed to overcome the protective effect.

MATERIAL AND METHODS

The following strains of tubercle bacilli were used in the experiments:

Streptomycin-susceptible tubercle bacilli (SMSTB): The Frankfurt strain (F strain), which was procured from the Research Institute for Tuberculosis and Leprosy, Sendai, and which has been shown to be susceptible to streptomycin concentrations of 0.5 γ per ml.

Streptomycin-resistant tubercle bacilli (SMRTB) (Endo strain): This strain was isolated by the writers in 1948 from a tuberculous patient (Endo, female, twenty-four years of age) who had been treated with 80 gm. of streptomycin. The strain is resistant to streptomycin concentrations greater than 1,000 γ per ml. of Dubos medium (16), and has been preserved in this laboratory for years without changing its resistance to streptomycin.

BCG: This material was also obtained from the Institute for Tuberculosis and Leprosy.

Paraffinized dead tubercle bacilli of F strain prepared according to the method of Couland (17).

Guinea pigs weighing 300 to 350 gm. were used. Initial inoculations were performed on the inner side of one thigh; reinoculations were performed on the other thigh. In other cases the flanks were used alternately. The inoculation was carried out subcutaneously.

In *Experiment III* the following method of intrapulmonic inoculation was utilized. The sharp point of a $\frac{1}{5}$ mm. caliber hypodermic needle was blunted and inserted into the lung parenchyma through the intact thoracic wall. A stylet of exactly the same length and internal diameter as the hypodermic needle was inserted through the latter, after having been immersed in a suspension of tubercle bacilli. Upon the completion of this operation, the stylet was removed first, followed by the needle. In this fashion, tubercle bacilli were deposited in a local area measuring approximately 3 mm., without contaminating surrounding lung parenchyma or entering venous channels. Confirmatory experiments using solid agar medium instead of pulmonary parenchyma illustrated the effectiveness of this method.

The culturing of tubercle bacilli from tissue was carried out as follows: a fixed amount of tissue was ground up, treated with 4 per cent sulfuric acid, and inoculated on solid medium of phosphata-ajinomoto-egg of Oka-Katakura (18) both with and without streptomycin. The concentration of streptomycin in this medium was between zero and 200 γ per ml. In some instances cultures were done on Oka-Katakura medium without streptomycin and the colonies were then tested by submerged growth in Dubos Tween[5]-albumin liquid medium (16) with 1 to 1,000 γ of streptomycin per ml.

In the accompanying tables the degree of bacterial growth has been indicated quantitatively in those instances in which it could be estimated. The signs −, ±, +, 2+, 3+, and 4+, respectively, refer to no growth, questionable growth, approximately 100 colonies, and growth occupying approximately one-third, two-thirds, and the entire slant of solid medium. In the case of Dubos liquid medium the plus signs refer respectively to no turbidity, uncertain turbidity, and turbidity after two and four days, one week and two weeks.

Experiments:

I (tables 1 and 2). Four groups of guinea pigs were inoculated with 0.001 to 0.05 mg. of streptomycin-susceptible tubercle bacilli (animals *Nos. 221*, *227*, and *228* were inoculated with material from the lung of a tuberculous rabbit as the initial inoculation). After intervals of one to eight months, 0.01 to 0.05 mg. of streptomycin-resistant tubercle bacilli were reinoculated. The animals were sacrificed between thirty-three and one hundred and ten days after reinoculation. Animal *No. 221* was autopsied two days after reinoculation.

TABLE 1

EXPERIMENT I. AUTOPSY FINDINGS IN GUINEA PIGS REINOCULATED WITH DIFFERENT QUANTITIES OF STREPTOMYCIN-RESISTANT TUBERCLE BACILLI

Groups	Quantity of Streptomycin-Resistant Tubercle Bacilli Used in Reinoculation	Quantity of Streptomycin-Susceptible Bacilli Used in First Inoculation	Designation of Animals by Numerical Order	Interval Between First and Second Inoculation	Days After Reinoculation	Macroscopic Findings																
						Site of First Inoculation	Site of Reinoculation	Lung	Liver	Spleen	Weight of Spleen	Lymph Nodes										Kidney
												Right Inguinal	Left Inguinal	Right Axillary	Left Axillary	Pulmonary Hilum	Mesenteric	Hepatic Hilum	Retroperitoneal	Cervical	
				months							*grams*											
1	0.01 mg.	0.001 mg.	351	1	75	Small* ulcer and abscess	Large† abscess	+	+	+	0.7	+	+	−	+	2+	+	+	+	±	−
			350	1	78	Ulcer and abscess	Large abscess	+	±	+	0.8	3+	−	−	+	2+	2+	2+	2+	−	−
			352	1	93	Abscess and crust	Large abscess and ulcer	3+	+	4+	1.2	3+	2+	−	+	+	+	+	+	+	−
			353	1	104	Ulcer and abscess	Abscess and ulcer	3+	+	4+	1.9	3+	2+	±	−	2+	2+	4+	+	2+	−
			349	1	110	Ulcer	Abscess	3+	2+	4+	2.6	3+	+	−	−	2+	2+	2+	+	+	−
2	0.05 mg.	0.05 mg.	314	1	42	Large ulcer	Small ulcer	+	+	3+	2.0	3+	2+	2+	−	4+	3+	3+	2+	+	−
			316	1	48	Large ulcer and abscess	Small ulcer	3+	−	3+	1.5	3+	3+	±	−	+	2+	3+	2+	+	−
			333	4	32	Rice-grain ulcer	Large abscess	3+	2+	3+	2.5	3+	2+	−	−	3+	+	3+	+	2+	−
			337	4	33	Rice-grain ulcer	Large abscess	3+	+	4+	1.3	+	±	−	−	+	+	+	±	+	±
			336	4	37	Rice-grain ulcer	Large abscess	2+	+	3+	2.1	3+	±	±	±	+	+	+	+	±	−
			340	4	37	Rice-grain ulcer	Large abscess	3+	4+	4+	8.1	3+	±	±	±	2+	2+	2+	2+	2+	−
			348	4	44	Rice-grain ulcer	Large ulcer	+	+	±	0.7	3+	+	±	±	+	2+	+	+	+	−
			338	4	51	Rice-grain ulcer	Small ulcer	2+	4+	4+	5.3	2+	2+	±	+	+	+	+	+	+	−
			332	4	75	Rice-grain ulcer	Large abscess	+	±	±	0.6	3+	2+	+	±	3+	2+	3+	3+	2+	−

3	0.1 mg.	0.1 cc. of lung extract from a tuberculous rabbit	221	8	2	Finger-tip ulcer	Large induration	3+	3+	3+	3.0	2+	+	2+	2+	+	2+	3+	3+	4+	–
			227	8	40	Finger-tip ulcer	Miliary ulcer	3+	3+	4+	2.7	2+	3+	3+	3+	+	+	4+	3+	+	–
			228	8	107	Finger-tip ulcer	Large ulcer	3+	3+	3+	4.5	2+	2+	+	+	2+	2+	3+	2+	2+	–
		0.001 mg.	330	1	51	Rice-grain ulcer and abscess	Large ulcer	±	+	3+	0.8	2+	2+	±	±	+	+	+	+	+	–
			331	1	66	Large ulcer and abscess	Large ulcer	2+	+	4+	1.9	3+	+	–	+	3+	+	2+	+	2+	–
4	0.5 mg.	0.05 mg.	326	2	46	Small abscess	Small abscess	3+	4+	4+	18.7	2+	+	2+	+	2+	2+	3+	3+	2+	–
			327	2	66	Finger-tip abscess	Finger-tip abscess	3+	3+	4+	16.0	2+	2+	2+	+	2+	+	2+	2+	2+	–

* Small—from less than half the size of a rice grain to a rice grain.
† Large—larger than two to three times the size of a rice grain.

	Organ	Lymph Node
–	No change	No change
±	Uncertain	Half the size of a rice grain up to a rice grain
+	A few tubercles	Two to three times larger than a rice grain
2+	Several tubercles	As big as a soya bean
3+	Middle degree of change	As big as a broad bean
4+	High degree of change	Bigger than a broad bean
5+	Entire organ filled with tuberculous lesions	

II (tables 3 and 4). Five groups of guinea pigs were injected with different kinds of tuberculous material:

Group 1: First inoculation—F strain, 0.005 mg.; *Group 2:* BCG, 5.0 mg.; *Group 3:* dead F strain in paraffin, 5.0 mg. These first three groups were reinoculated with 0.001 mg. of Endo strain (SM*R*TB).

Group 4: The same dose of Endo strain without preliminary inoculation.

Group 5: BCG alone.

III (tables 5 and 6). Two groups of guinea pigs were utilized:

Group 1: Inoculation of 0.01 mg. of streptomycin-susceptible tubercle bacilli (F strain) in the right thigh. Eighty-three days later, reinoculation with streptomycin-resistant tubercle bacilli (Endo strain) within the lung parenchyma.

Group 2: Intrapulmonic inoculation of streptomycin-resistant tubercle bacilli (Endo strain) without preliminary inoculation.

RESULTS

Experiment I: In table 1 are shown the autopsy findings of the animals in this experiment in which the inoculation of streptomycin-susceptible tubercle bacilli preceded, by various periods, the inoculation with streptomycin-resistant tubercle bacilli. Some changes were observed in almost all of the animals at the site of the first and of the second inoculations. These consisted primarily of abscess formation or ulceration, or both. There was some regional lymph node involvement of a tuberculous nature. The mesenteric, retroperitoneal, hepatic, and mediastinal lymph nodes were involved to a variable extent. The liver, lung, and spleen also showed tuberculous changes. The degree of changes appeared to correspond largely to the time interval between inoculations. There was no correlation between numbers of reinoculated tubercle bacilli and anatomic changes.

The results of cultures of tubercle bacilli from tissues obtained are shown in table 2.

Group 1 was inoculated initially with 0.001 mg. of streptomycin-susceptible tubercle bacilli and reinoculated with 0.01 mg. of streptomycin-resistant tubercle bacilli. On streptomycin-free medium, culture of almost all organs and lymph nodes for tubercle bacilli was positive in all 5 animals. In medium containing streptomycin, however, tubercle bacilli could be demonstrated only in the actual reinoculation site and in regional lymph nodes (left inguinal lymph nodes). The only exceptions to the latter case were in animals *Nos. 351* and *349* from which were demonstrated three colonies from the left axillary lymph node and four colonies from the spleen, respectively. Animal *No. 349* also was autopsied one hundred and ten days after reinoculation. When larger numbers of bacilli were reinoculated, as in *Groups 2* to *4*, there was more visceral involvement than in *Group 1*, in which a smaller number of bacilli were used.

It may be concluded from this experiment that the initial inoculation resulted in enough immunity to prevent spread by reinoculated tubercle bacilli, although this was not completely true when large numbers were used. It may be mentioned here that it was repeatedly verified in preliminary experiments that no growth was found on streptomycin-containing medium if the animals had been inoculated solely with streptomycin-susceptible tubercle bacilli.

TABLE 2

EXPERIMENT I. RESULTS OF CULTURES OF TUBERCLE BACILLI FROM TISSUES

Groups	Quantity of Reinoculated Bacilli	Quantity of Bacilli Used in First Inoculation	Designation of Animals by Numerical Order	Interval Between First and Second Inoculation	Days After Reinoculation	Streptomycin-Free Medium																Streptomycin-Containing Medium (100 γ per ML)																
						Site of First Inoculation	Site of Reinoculation	Right Inguinal Lymph Nodes	Right Popliteal Lymph Nodes	Left Inguinal Lymph Nodes	Left Popliteal Lymph Nodes	Right Axillary Lymph Nodes	Left Axillary Lymph Nodes	Mesenteric Lymph Nodes	Retroperitoneal Lymph Nodes	Lymph Nodes of the Liver Hilum	Lymph Nodes of the Pulmonary Hilum	Lung	Liver	Spleen	Kidney	Site of First Inoculation	Site of Reinoculation	Right Inguinal Lymph Nodes	Right Popliteal Lymph Nodes	Left Inguinal Lymph Nodes	Left Popliteal Lymph Nodes	Right Axillary Lymph Nodes	Left Axillary Lymph Nodes	Mesenteric Lymph Nodes	Retroperitoneal Lymph Nodes	Lymph Nodes of the Liver Hilum	Lymph Nodes of the Pulmonary Hilum	Lung	Liver	Spleen	Kidney
				months																																		
1	0.01 mg.	0.001 mg.	351	1	75	5	9	29		25			30		28	3		3	1	3+		–	12	–		14			3		–	–			–	–		
			350	1	78	2+	2+	18		4			–		18	10		16	2	2+		–	2+	–		2					–	–			–	–		
			352	1	93	–	3+	120		20			–		28	2+		88	12	100		–	2+	–		10					–	–			–	–		
			353	1	104	2	2+	2+		+			–		6	100		+	+	2+		–	2+	–		36					–	–			–	–		
			349*	1	110	8	2+	40		6			2		10	2+		110	2+	2+		–	2+	–		–					–	–			–	4		
2	0.05 mg.	0.05 mg.	314*	1	42	4+	4+	4+	4+	4+	4+	4+		4+			4+	4+				–	4+	–	–	4+	4+			2+			2+	2+		4+	
			316	1	48	4+	4+	4+		4+	4+				4+	4+		4+				–	4+	2+	–	4+	4+				2+	2+		+		4+	
			333	4	32	4+	4+	3+		3+				70	3+	3+	3+	38	4+	10	3+	2+	–	3+	–	–	–		–	–	–	–	–	–	–	18	–
			337	4	33	3+	3+	3+		3+		2+	3+	2+	3+	7	26	25	3+	4+	2+	–	3+	–		3+		–	18	–	20	–	–	–	–	22	–
			336*	4	37	3+	3+	3+	3+						5	4+		24		40		–	3+	–	–						–	–			–	–		
			340	4	37	4+	4+	4+		4+		4+	4+	4+	4+	4+	4+	4+	4+	4+			4+	–	–	4+	4+	–	4+	–	–	–	–	–	–	–	–	–
			348	4	44	3	150	150	10	10		55	35		39		27	1	5	52	100	–	8	–	–	–				–	–	–	–	–	–		
			338*	4	51	3+	3+	3+	82	138	65		105		150	3+	4+	120	50	3+			3+	30		16	13							+	+	–	
			332*	4	75	3+	4+	3+	4+	4+	4+	4+		4+	4+	4+	4+	4+	4+	4+	4+		–	3+	4+	–	4+	4+	–			–	–	3+	2+	2+	2+	3+	
3	0.1 mg	†	221*	8	2	4+	4+	3+	3+	3+	3+	3+	4+	4+				4+	4+	4+	+	–	4+	–		–			–	–			–	–	–		
			227	8	40	4+	4+	4+	4+	4+	3+	4+	4+	4+	3+	4+	4+	4+	4+	4+		–	4+	2+	2+	4+	3+	+	4+	–	+	2+		–	–	3+	
			228	8	107	4+	4+	3+	4+	4+	4+	4+	4+	4+	3+	3+	2+	2+	2+	3+	4+	–	4+			4+	4+	3+	3+	–	–	–	–	–	–	–	
		0.001	330	1	51	3+	3+	3+		3+		150	3+	24	150		3+	120	3+	3+		–	3+	4		40		1	–	–	–	–	–	–	–	–	
		mg.	331	1	66	4+	4+	4+	4+	4+	4+	4+	4+	3+	3+	3+	3+		3+	3+	3+	–	80	3	–	75		–	10	–	–	–	–	6	–	–	–
4	0.5 mg.	0.05 mg.	326*	2	46	4+	4+	4+		4+	4+	4+	4+	4+	3+	4+	3+	4+	3+	2+	3+	–	4+	3+		4+	4+	2+		2+	–	–	+	2+	–	2+	3+
			327*	2	66	4+	4+	4+	3+	4+	3+	3+	4+	4+		3+	3+	4+	4+	4+	3+	–	4+		2+	4+	3+	3+	+	+		2+	2+	–	–	–	3+

* Cultured by indirect method.

† Extract of tuberculous lung.

TABLE 3

EXPERIMENT II. AUTOPSY FINDINGS IN GUINEA PIGS SUBJECTED TO DIFFERENT KINDS OF PRIMARY PROCEDURES

Groups*	Designation of Animals by Numerical Order	Days After Reinoculation	Body Weight			Tuberculin Reaction Before Autopsy (Induration)	Macroscopic Findings														
							Sites		Organs				Lymph Nodes								
			Beginning	At the Time of Reinoculation	†At the Time of Autopsy		First Inoculation	Reinoculation	Lung	Liver	Spleen	Weight of Spleen	Right Inguinal	Left Inguinal	Right Axillary	Left Axillary	Pulmonary Hilum	Mesenteric	Hepatic Hilum	Hypogastric
			grams	*grams*	*grams*							*grams*								
1	354	7	330	350	365	25 × 25	Soya-bean ulcer	Soya-bean induration	+	±	3+	0.5	+	2+	±	+	+	±	+	+
First inoculation: streptomycin-susceptible tubercle bacilli (Frankfurt strain), 0.005 mg.	355	12	360	385	425	20 × 20	Soya-bean ulcer	Soya-bean abscess	2+	+	4+	0.9	2+	3+	+	3+	3+	3+	3+	3+
	356	20	325	330	350	25 × 25	Pea ulcer	Soya-bean abscess	+	+	4+	0.7	+	2+	+	2+	2+	+	+	+
Reinoculation: streptomycin-resistant tubercle bacilli (Endo strain), 0.01 mg.	357	28	310	330	345	18 × 18	Ulcer and crust	Soya-bean abscess	2+	2+	4+	1.8	2+	3+	+	2+	2+	+	2+	2+
	358	35	360	370	410	25 × 25	Ulcer and crust	Soya-bean abscess	2+	2+	4+	0.6	3+	3+	+	2+	3+	+	2+	2+
	359	45	280	310	320	20 × 22	Ulcer and crust	Pea abscess	3+	2+	4+	2.3	3+	3+	+	2+	+	2+	2+	2+
	360	52	385	410	400	15 × 15	Crust	Pea abscess	3+	3+	4+	4.1	3+	3+	+	2+	4+	+	2+	2+
2	362	7	380	395	400	10 × 10	Scar	Abscess and crust	–	–	–	1.1	±	±	–	–	–	–	–	–
First inoculation: BCG, 5.0 mg.	363	12	295	300	322	25 × 30	Scar	Soya-bean abscess	–	–	–	0.4	+	±	–	–	–	–	–	–
Reinoculation: streptomycin-resistant tubercle bacilli (Endo strain), 0.01 mg.	368	21	600	630	620	33 × 33	Scar	Rice-grain abscess and crust	±	–	±	0.7	+	±	–	–	+	+	+	–
	365	28	440	430	540	22 × 21	Scar	Soya-bean abscess and crust	–	–	–	0.6	2+	±	±	±	+	+	+	±
	366	33	295	360	450	20 × 20	Scar	Soya-bean abscess and crust	±	–	3+	0.5	+	±	–	±	2+	±	±	3+
	367	42	300	320	300	22 × 22	Scar	Soya-bean abscess and crust	±	–	+	0.8	2+	+	–	±	2+	±	±	2+
	369	51	410	430	405	20 × 20	Scar	Soya-bean abscess and crust	–	–	–	0.6	+	±	–	–	2+	2+	2+	±

3	398	7	455	470	400	24 × 19	Finger-tip abscess and ulcer	Abscess	−	−	−	0.3	±	2+	−	2+	−	−	−	−
First inoculation: paraffin-embedded dead tubercle bacilli, 5.0 mg.	397	14	430	450	460	30 × 30	Finger-tip abscess and ulcer	Soya-bean abscess	+	+	2+	1.1	±	+	−	+	+	+	+	−
	399	21	380	400	410	20 × 20	Finger-tip abscess and ulcer	Soya-bean abscess	−	−	−	0.6	+	2+	−	2+	±	−	−	−
Reinoculation: streptomycin-resistant tubercle bacilli (Endo strain), 0.01 mg.	393	28	515	550	660	25 × 30	Finger-tip abscess and ulcer	Finger-tip abscess	±	±	2+	0.8	2+	±	−	2+	2+	−	−	+
	400	35	500	490	470	25 × 30	Finger-tip abscess and ulcer	Abscess	−	−	+	0.9	2+	2+	−	2+	+	−	−	+
4	381	7	295		320		Induration		−	−	−	0.4	−	−	−	−	−	−	−	−
Simple inoculation: streptomycin-resistant tubercle bacilli (Endo strain), 0.01 mg.	382	14	290		310		Soya-bean abscess		−	−	±	0.9	2+	−	−	−	±	±	±	±
	383	22	305		350	30 × 30	Finger-tip abscess		2+	+	4+	1.0	3+	±	±	±	2+	+	2+	2+
	385	34	300		330	25 × 20	Soya-bean abscess and ulcer		±	±	4+	0.4	2+	+	+	+	+	2+	+	2+
	386	42	310		330	23 × 20	Finger-tip abscess and ulcer		2+	2+	4+	1.9	2+	2+	+	+	2+	2+	+	+
	387	52	380		420	25 × 25	Finger-tip abscess and ulcer		+	+	4+	0.7	3+	+	2+	+	2+	2+	2+	2+
	388	62	370		400	20 × 20	Finger-tip abscess and ulcer		4+	4+	4+	33.0	3+	2+	2+	2+	2+	2+	2+	2+
5	371	14	305		300		Finger-tip ulcer		−	−	−	0.3	−	±	−	−	−	−	−	−
BCG alone, 5.0 mg.	373	21	180		235	15 × 15	Pea ulcer		±	−	−	0.4	−	+	−	±	−	−	−	−
	374	28	310		320	20 × 20	Crust		±	−	−	0.8	−	+	−	±	−	−	−	−
	375	30	190		225	15 × 15	Scar		−	−	−	0.5	−	±	−	±	−	−	−	−
	376	32	250		270	25 × 23	Scar		−	−	−	0.4	−	±	−	±	−	−	−	−
	377	49	340		370	25 × 25	Scar		−	−	−	0.6	−	±	−	±	−	−	−	−

* Reinoculation was done six weeks after first inoculation.

Experiment II: The purpose of this experiment was to demonstrate the protective action of BCG or paraffinized dead tubercle bacilli against subsequent inoculation. The autopsy findings are demonstrated in table 3. The changes at the site of the first inoculation differed according to the type of material used. In *Groups 1* and *4*, in which virulent strains were used for the preliminary inoculation, the site usually showed abscess or ulcer formation. In *Groups 2* and *5*, which received BCG, the inoculation sites most commonly formed scar formation. In *Group 3*, using paraffin-embedded dead tubercle bacilli, abscess formation with small ulceration occurred at the site of the first inoculation. Almost all the animals of *Groups 1*, *2*, and *3* had abscess formation with or without ulceration at the site of the second inoculation.

With respect to these autopsy findings, similar changes were observed in animals of *Groups 1* and *4*, both of which had received virulent tubercle bacilli at the time of first inoculation (*Group 1*, the F strain (SM*S*TB) and *Group 2*, Endo strain (SM*R*TB)). As can be seen from table 3, the changes in *Group 1* are more extensive than in *Group 4*. It may be possible to attribute this, not to the difference in the susceptibility of tubercle bacilli to streptomycin, but to the time interval between the first inoculation and autopsy. Thus, *Group 1* was allowed to survive six weeks longer after primary inoculation than *Group 4*. *Groups 2*, *3*, and *5* showed less extensive changes than *Groups 1* and *4*.

These findings suggest that the changes in *Groups 1* and *4* are due to the first rather than the second inoculation.

In *Group 1* (table 4), tubercle bacilli (SM*S*TB) inoculated at the primary injection were recovered from all organs, while bacilli introduced at reinoculation (SM*R*TB) were confined to the site of reinoculation and draining lymph nodes. In *Group 4*, which received only streptomycin-resistant tubercle bacilli at the same time as reinoculation of the other groups was carried out, bacilli were obtained from all organs on streptomycin-free as well as on streptomycin-containing media. It is natural that the streptomycin-resistant tubercle bacilli grow on both media. In *Group 5*, which received BCG strain alone, bacilli were recovered mostly from the site of inoculation and to a much less extent from regional lymph nodes. Only 2 animals of 6 demonstrated a few colonies in material taken from the spleen and the left axillary lymph nodes, respectively. BCG at the site of inoculation decreased rapidly and, in animal *No. 377*, no bacilli were detectable forty-nine days after inoculation. In *Group 2*, which was inoculated initially with BCG and subsequently with streptomycin-resistant tubercle bacilli, it may be seen that the latter bacilli (SM*R*TB) were confined mainly to the site of reinoculation and draining lymph nodes. In a few animals, however, after a lapse of four weeks or more following reinoculation, bacilli could be recovered from spleen, lung, and mesenteric lymph nodes. If this group is compared with *Group 1* and *Group 4*, it may be seen that the protecting power of BCG given at the primary inoculation was much weaker than it was with the virulent strain. The results in *Group 3* showed that the paraffinized dead F strain of tubercle bacilli was also able to produce immunity against the inoculation of virulent tubercle bacilli but was still weaker than BCG.

Experiment III: In contrast to *Experiments I* and *II*, reinoculation in this group was made directly into the lung parenchyma with the method described above. The autopsy findings are demonstrated in table 5. In *Group 2*, the Endo strain (SM*R*TB) alone was inoculated into the right lung on the same day as reinoculation of *Group 1*. The number of lesions at the site of inoculation in the right lung in this group was rather small. The left lung also had occasional lesions. All animals developed extensive changes in the spleen after a lapse of more than nineteen days. The same applied to the liver after eighty-four and ninety days. Swelling of the lymph nodes was not remarkable except in the mediastinal areas. The lesions in *Group 1*, which had received preliminary subcutaneous inoculation eighty-three days before intrapulmonic injection, were much more intensive than those in *Group 2*. The widespread distribution of lesions in this group was probably due to the long interval between the first inoculation and autopsy. The lesions at the sites of the first inoculation and the draining lymph nodes were similar to those observed in *Experiment I*. The intrapulmonic reinoculation site showed more restricted changes than those of *Group 2*.

The bacteriologic findings in this group are summarized in table 6. In *Group 1*, bacilli were recovered from all organs and lymph nodes on streptomycin-free medium in abundant quantities, while the streptomycin-containing medium grew only a few colonies. However, if one compares this group with *Group 1* of *Experiment II*, in which the animals received hypodermic subcutaneous injections, both for preliminary inoculations and reinoculations, the growth of bacilli from the viscera of this group is much more extensive on streptomycin-containing medium. In *Group 2*, culture on streptomycin-containing medium in some animals was unsuccessful.

It may be concluded from this experiment that the subcutaneous preliminary inoculation of virulent tubercle bacilli can impair the dissemination of organisms inoculated into the lung parenchyma. However, this defense is much weaker than that set up against subcutaneous reinoculation.

DISCUSSION

The question under study was whether streptomycin-resistant tubercle bacilli are suited for use in the study of reinfection tuberculosis. It is generally accepted that streptomycin-resistant tubercle bacilli represent a mutation product selected by the drug. As shown above, streptomycin-resistant tubercle bacilli can readily be differentiated from streptomycin-susceptible tubercle bacilli, which grow only on streptomycin-free media, whereas the former can be observed on streptomycin-containing media.

It has recently been suggested that radioactive material may be useful for labeling tubercle bacilli. This appears to be an inadequate method for an experiment such as this because of the duration of the half-life of the isotope as well as its increased dilution with bacillary multiplication.

The classic experiments of Koch (1), Hamburger (19), and Römer (20) demonstrated that initial inoculation with tubercle bacilli resulted in dissemination of the entire body in a comparatively short time. Krause (3), Willis (4), Lurie (10), and Dahl (12) confirmed these observations by the use of culture methods.

TABLE 4

EXPERIMENT II. RESULTS OF CULTURES OF TUBERCLE BACILLI FROM TISSUES

Groups	Designation of Animals by Numerical Order	Days After Reinoculation	Streptomycin-Free Medium										Streptomycin-Containing Medium (200 γ per ML)									
			Site of First Inoculation	Site of Reinoculation	Right Inguinal Lymph Nodes	Left Inguinal Lymph Nodes	Left Axillary Lymph Nodes	Mesenteric Lymph Nodes	Lymph Nodes of the Pulmonary Hilum	Lung	Liver	Spleen	Site of First Inoculation	Site of Reinoculation	Right Inguinal Lymph Nodes	Left Inguinal Lymph Nodes	Left Axillary Lymph Nodes	Mesenteric Lymph Nodes	Lymph Nodes of the Pulmonary Hilum	Lung	Liver	Spleen
1	354	7	2+	3+	4	3+	8	–	46	2	6	3+	–	3+	–	–	–	–	–	–	–	–
First inoculation: Frankfurt strain, 0.005 mg.	355	12	2+	3+	28	33	4	–	74	7	6	3+	–	3+	–	–	–	–	–	–	–	–
Reinoculation: Endo strain, 0.01 mg.	356	20	2+	2+	11	40	2+	21	2		7	2+	–	2+	4	–	–	–	–	–	–	–
	357	28	2+	3+	2+	3+	2+	2+	2+	26	13	3+	–	3+	28	–	–	–	–	–	–	–
	358	35	2	2+	3	3+	10	35	14	2+	39	2+	–	2+	3	–	–	–	–	–	–	–
	359	45	2+	2+	2+	2+	3+	2+	2+	20	40	80	–	2+	15	–	–	–	–	–	–	–
	360	52	1	3+	6	23	4+	2	9	3+	10	3+	–	3+	6	–	–	–	–	–	–	–
2	362	7	3	3+	–	6	–	–	–	–	–	–	–	3+	1	–	–	–	–	–	–	–
First inoculation: BCG, 5.0 mg.	363	12	2+	4+	5	–	–	–	–	–	–	–	–	3+	8	–	–	–	–	–	–	–
Reinoculation: Endo strain, 0.01 mg.	368	21	2+	4+	22	–	–	–	–	–	–	–	–	3+	25	–	–	–	–	–	–	–
	365	28	–	4+	3+	–	–	1	–	–	–	4	–	4+	3+	–	–	–	–	–	–	4
	366	33	–	4+	22	–	–	–	–	–	–	–	–	4+	25	–	–	–	–	–	–	–
	367	42	–	4+	3+	–	–	10	–	–	–	1	–	4+	3+	–	–	18	–	–	–	–
	369	51	–	4+	3+	–	–	2	–	2+	–	2+	–	4+	2+	–	–	3	–	+	–	2+
3	398	7	–	3+	–	–	–	–	–	–	–	–	–	2+	–	–	–	–	–	–	–	–
First inoculation: Paraffin-embedded dead bacilli, 5.0 mg.	397	14	–	3+	33	–	–	11	2	6	1	18	–	4+	13	1	–	8	3	2	–	22
Reinoculation: Endo strain, 0.01 mg.	399	21	–	4+	3+	–	–	–	–	–	–	–	–	3+	3+	–	–	–	–	–	–	–
	393	28	–	4+	3+	–	–	8	–	–	–	12	–	3+	2+	–	–	20	–	–	–	14
	400	35	–	4+	4+	–	–	–	–	3	–	3+	–	4+	3+	–	–	–	–	15	–	2+

4	381	7	3+		23	–	–	–	–	–	–	–	3+		52	–	–	–	–	–	–	–
(Control)	382	14	4+		2+	–	1	6	2	–	10	2+	4+		2+	–	–	4	–	–	6	2+
Endo strain, 0.01 mg. (no preliminary treatment)	383	22	4+		4+	3	50	2+	2+	2	1	2+	4+		4+	2	38	2+	22	3	–	2+
	385	34	4+		3+	3+	2+	6	2+	–	11	3+	4+		3+	3+	2+	6	32	2	12	3+
	386	42	4+		3+	3+	18	58	3+	2+	2+	4+	4+		3+	3+	3	19	3+	2+	2+	4+
	387	52	4+		4+	–	–	–	3+	2	19	36	4+		3+	–	–	–	3+	3	8	23
	388	62	4+		3+	3+	2+	2+	4+	4+	4+	4+	4+		4+	3+	3+	2+	4+	4+	4+	4+
5	371	14	3+		–	4	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
(Control)	373	21	2+		–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
BCG, 5.0 mg. (no preliminary treatment)	374	28	2+		–	50	2	–	–	–	–	5	–		–	–	–	–	–	–	–	–
	375	30	20		–	4	–	–	–	–	–	1	–		–	–	–	–	–	–	–	–
	376	32	1		–	2	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
	377	49	0		–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–

TABLE 5

EXPERIMENT III. AUTOPSY FINDINGS IN GUINEA PIGS SUBJECTED TO INTRAPULMONIC REINOCULATION

Groups*	Designation of Animals by Numerical Order	Quantity of Tubercle Bacilli Used in First Inoculation	Days After First Inoculation	Days After Reinoculation	Body Weight		Site of First Inoculation	Site of Reinoculation	Lung		Pulmonary Hilar Lymph Nodes		Liver	Spleen		Kidney	Lymph Nodes						
					Beginning	End			Right	Left	Right	Left		Lesions	Weight		Right Inguinal	Left Inguinal	Right Axillary	Left Axillary	Mesenteric	Hepatic Hilum	Retroperitoneal
					grams	*grams*									*grams*								
1	411♂	0.01 mg.		4	540	350	Soya-bean ulcer and abscess	?	2+	2+	2+	+	4+	5+	12.5	−	2+	+	+	+	+	+	+
	412♀	0.01 mg.		16	420	430	Soya-bean ulcer and abscess	Right lower lobe, half a rice grain	2+	2+	+	+	3+	4+	5.5	−	3+	−	±	−	±	2+	2+
	413♀	0.01 mg.		19	520	380	Soya-bean ulcer and abscess	?	+	+	+	+	2+	4+	1.1	−	3+	+	+	±	+	2+	2+
	414♀	0.01 mg.		30	600	400	Pea ulcer and abscess	Right middle lobe, miliary	2+	2+	+	+	3+	4+	4.5	−	3+	+	−	−	+	2+	−
	415♂	0.01 mg.		34	540	480	Soya-bean abscess	Right middle lobe, half a rice grain	2+	−	2+	2+	3+	3+	3.0	−	3+	+	−	−	−	−	−
	416♀	0.01 mg.		48	580	400	Indian-bean ulcer and abscess	?	2+	2+	2+	2+	3+	4+	4.6	−	3+	±	+	−	−	3+	+
	417♂	0.01 mg.		60	600	500	Indian-bean ulcer and abscess	?	+	−	2+	2+	2+	3+	0.9	−	2+	2+	−	−	−	2+	3+
	418♀	0.01 mg.		65	460	500	Indian-bean crust and abscess	?	2+	2+	2+	2+	3+	3+	1.7	−	3+	±	±	±	+	2+	2+
	419♀	0.01 mg.		72	400	460	Soya-bean abscess	?	2+	−	2+	2+	3+	4+	2.3	−	3+	−	−	−	2+	2+	+
	420♀	0.001 mg.		76	450	540	Soya-bean ulcer and abscess	?	2+	+	2+	+	2+	5+	4.2	−	3+	±	±	±	2+	2+	3+

Hypodermic inoculation

	421 ♀	0.01 mg.		78	500	560	Soya-bean ulcer and abscess	Right upper lobe, half a rice grain	4+	4+	2+	+	4+	5+	20.8	−	3+	3+	+	+	2+	2+	3+
	422 ♂	0.001 mg.		110	700	560	Soya-bean ulcer	?	5+	5+	+	+	4+	5+	6.5	−	3+	−	+	−	2+	3+	−
2	426 ♂	Intrapulmonic inoculation ca. 2,250 tubercle bacilli	13		440	420	?		±	±	±	±	−	−	0.4	−	−	−	−	−	−	−	−
	427 ♀		13		490	480	Right lower lobe, miliary		+	−	−	−	−	−	0.3	−	−	−	−	−	−	−	−
	428 ♀		15		300	240	Right lower lobe, miliary		+	−	±	±	−	−	0.3	−	−	−	−	−	−	−	−
	429 ♀		16		400	400	Right upper lobe, miliary		+	+	+	−	−	+	0.8	−	−	−	±	−	−	−	−
	430 ♂		19		320	280	Right middle lobe, wedge		+	−	+	−	−	4+	0.5	−	−	−	±	±	±	±	−
	431 ♂		19		340	300	Right lower lobe, wedge		+	+	+	±	−	4+	0.4	−	±	±	±	−	−	−	−
	432 ♂		19		230	220	Right lower lobe, wedge		2+	+	+	±	−	4+	0.3	−	−	−	−	−	−	2+	−
	433 ♂		19		360	380	?		−	−	+	+	−	4+	0.4	−	−	−	+	±	±	−	−
	434 ♂		19		310	250	Right lower lobe, half a rice grain		+	−	+	±	−	4+	0.3	−	−	−	−	−	−	−	−
	435 ♂		21		310	350	Right middle lobe, miliary		+	+	+	±	−	4+	0.2	−	−	−	±	±	−	−	−
	436 ♀		21		440	420	Right lower lobe, miliary		+	−	+	−	+	4+	0.4	−	−	−	−	−	−	−	−
	437 ♂		21		490	500	Right lower lobe, miliary		+	−	+	−	−	3+	0.8	−	−	−	±	±	−	−	−
	438 ♂		84		380	500	?		+	+	+	+	4+	4+	1.4	−	−	−	−	−	−	−	−
	439 ♂		98		350	400	Wedge as large as half a rice grain		3+	3+	+	+	4+	4+	36.0	−	±	±	2+	+	+	+	±

* Reinoculation was done intrapulmonically eighty-three days after first inoculation.

TABLE 6

EXPERIMENT III. RESULTS OF CULTURES OF TUBERCLE BACILLI FROM TISSUES

Groups	Designation of Animals by Numerical Order	Days After First Inoculation	Days After Reinoculation	Streptomycin-Free Medium										Streptomycin-Containing Medium (100 γ per Ml.)									
				Right Lung	Left Lung	Lymph Nodes of the Right Pulmonary Hilum	Lymph Nodes of the Left Pulmonary Hilum	Liver	Spleen	Right Inguinal Lymph Nodes	Left Inguinal Lymph Nodes	Mesenteric Lymph Nodes	Retroperitoneal Lymph Nodes	Lymph Nodes of the Liver Hilum	Right Lung	Left Lung	Lymph Nodes of the Right Pulmonary Hilum	Lymph Nodes of the Left Pulmonary Hilum	Liver	Spleen	Right Inguinal Lymph Nodes	Left Inguinal Lymph Nodes	Mesenteric Lymph Nodes	Retroperitoneal Lymph Nodes	Lymph Nodes of the Liver Hilum
1	411		4	4+	4+	4+	4+	2+	3+	+	1	82	78	60	+	5	–	–	–	–	–	–	–	–
First inoculation: Frankfurt strain, subcutaneously—streptomycin-susceptible	412		16	3+	3+	2+	2+	44	3+	2+	–	10	19	28	+	–	–	–	–	–	–	–	–	–
	413		19	4	30	60	56	48	3+	3+	–	21	–	40	–	10	2	12	–	+	–	–	–	–
	414		30	49	2+	13	56	–	+	175	64	+	25	100	5	6	1	42	–	22	–	–	1	13	4
	415		34	+	40	45	75	3+	3+	4+	3+	5	+	20	+	72	7	16	2+	2+	–	–	–	–	3+
	416		48	3+	3+	2+	2+	30	4+	2+	+	–	–	3+	10	+	+	50	1	4+	3+	23	–	–	+
Reinoculation: Endo strain, intrapulmonically—streptomycin-resistant	417		60	+	5	2+	26	18	9	100	+	+	–	3+	26	6	+	70	1	+	–	100	–	–	–
	418		65	24	50	12	40	3+	4+	2+	–	16	16	2+	–	5	10	2	2	26	–	–	11	–	–
	419		72	+	+	2+	16	3+	3+	3+	39	–	+	3+	–	–	1	–	–	+	–	37	–	12	–
	420		76	3+	2+	4+	4+	4+	4+	100	2+	–	+	3+	+	+	+	+	3	4+	–	–	–	–	4
	421		78	4+	4+	3+	3+	4+	4+	4+	3+	2+	+	3+	100	2+	+	+	19	3+	–	–	–	–	–
	422		110	3+	3+	59	2+	100	4+	3+	2+	8		3+	6	–	–	–	+	2+	–	–	2		–
2	426	13		3+	2+	2+	+	+	4+	31	8	–	70	42	3+	2+	2+	+	+	3+					23
Endo strain, intrapulmonically (no preliminary treatment)	427	13		3+	39	2+	28	118	4+	130	–	4+	–	2+	3+	+	2+	18	+	4+	31	–	100		
	428	15		300	70	3+	2+	28	200	10	2	250	26	48	3+	+			18	2+	15	4	–	10	
	429	16		70	40	3+	2+	+	4+	150	3+	3+	12	3+	+	+	3+	2+	100	4+	150	3+			
	430	19		2+	31	2+	+	4+	3+	4+	80	4+	4+	4+	+	14	2+	+	4+	4+	4+	11	4+	4+	4+
	431	19		4+	+	3+	+	50	3+	200	3+	3+	42	4+	3+	+	2+	2+	36	3+	2+				
	432	19		2	2+	3+	2+	3+	4+	+	25	4+	2+	+	+		3+		3+	4+					
	433	19		100	–	3+	28	+	3+						+	–	3+	2+	+	3+	8	–	100	–	–
	434	19		3+	+	100	2+	2	2+						3+	+	2+								
	435	21		29	18	2+		80	4+	6	–	3+	–	–											
	436	21		3+		3+		63		50		2+	69												
	437	21		+	–	–	–	3+	3+	–	–	4+	–	2											
	438	84		2+	36	3+	72	18	3+	+	4	2+	–	2+	2+	14	3+	+	42	3+	9	–	2+	–	2+
	439	98		3+	2+	4+	2+	150	4+	28	2	4+	2+	3+	3+	2+	4+	2+	3+	4+	9	17	4+	2+	3+

The present experiments showed similar results. On subcutaneous inoculation of virulent tubercle bacilli (both streptomycin-susceptible and streptomycin-resistant), lesions developed in various organs fourteen days after exposure. Twenty-two days after inoculation, tubercle bacilli could be isolated from all organs. When the intrapulmonic route was utilized, cultures taken from animals within thirteen days demonstrated abundant growth. In the case of primary BCG inoculation, the situation differed in that the organisms were limited to the inoculation site and regional lymph nodes for a much longer period of time. Only occasional colonies were recovered from the spleen and distant lymph nodes.

It is generally accepted that primary infection of tuberculosis confirms immunity. Koch, Hamburger, Römer, and Calmette and Guérin (21) demonstrated this observation; and Boquet and Valtis, as well as Lurie, confirmed these findings using tubercle bacilli, both of human and bovine origin. Lurie (10) quoted Selter, Kalbfleisch, and Hedval as suggesting that reinoculated tubercle bacilli remain for a long period of time at the site of reinoculation. In his experiment, Lurie performed intravenous reinoculation on rabbits and stated that, "In the presence of sufficient immunity, such as occurs with the persistence of extensive primary lesions, the bacilli of reinfection may be completely destroyed . . . ." The results of his experiments seem to vary with the species of animals and virulence of bacilli used.

It is well known that human tubercle bacilli are less virulent for rabbits, and bovine types are highly virulent. Boquet and Valtis, Lurie, Boquet and Laporte, and Dahl, employing bacilli of the human and bovine varieties, used different types of bacilli for primary and secondary inoculation. By this method they attempted to differentiate the types of organisms found. This method, however, appears to have been impractical, and the present experiments are more convincing. Virulent human tubercle bacilli (streptomycin-resistant), when reinoculated subcutaneously into the tuberculous animal, remain for a long time at the site of reinfection and regional lymph nodes. In this regard they somewhat resemble less virulent organisms, such as BCG, when these are inoculated for the first time. However, if the same strain of bacilli is used in high doses, it passes through the barrier of immunity and becomes disseminated throughout the body.

With regard to the fate of reinoculated organisms when introduced subcutaneously, they are arrested at the inoculation site and regional lymph nodes, although they disseminate readily if introduced into the lungs. It may be stated parenthetically that many fewer organisms were introduced intrapulmonically than subcutaneously and that the use of pressure was avoided so as not to force bacilli into venous channels. The technical factor appears to be not without practical significance if one thinks of the natural mode of infection of tuberculosis.

Incidentally, it must be added that a virulent strain of tubercle bacilli is necessary at the time of primary infection to develop adequate immunity against a high dose of reinoculated virulent bacilli. BCG and paraffin-embedded dead bacilli of a virulent strain, although capable of creating immunity, are not nearly as effective in this regard as virulent organisms. In these cases subcutaneously reinoculated virulent organisms sometimes pass through the barrier of the reinoculation site and the draining lymph nodes.

SUMMARY

Streptomycin-resistant tubercle bacilli may be used as labeled organisms in experiments on reinfection tuberculosis. The use of these streptomycin-resistant tubercle bacilli is based on the ability to differentiate streptomycin-susceptible and streptomycin-resistant organisms on culture media. The streptomycin-susceptible organisms grow only on culture media free of streptomycin, whereas resistant organisms are able to grow on drug-free medium as well as on medium containing streptomycin.

If initial inoculation with virulent tubercle bacilli is performed, subcutaneously reinoculated organisms are arrested at the site of reinoculation and draining lymph nodes. Only in cases of very large doses of reinoculated bacilli is it possible to demonstrate organisms in distant organs.

The BCG strain and paraffin-embedded dead tubercle bacilli of a virulent strain, although they produce similar immunity, do so to much less an extent than virulent organisms.

When reinoculation takes place in the lungs, the bacilli are disseminated throughout the body, even though much smaller doses are used.

SUMARIO

De los Problemas de la Reinfección en la Tuberculosis. Cabe Usar los Bacilos Tuberculosos Estreptomicinorresistentes como Bacilos Rotulados en los Experimentos de Reinoculación?

Cabe usar bacilos tuberculosos estreptomicinorresistentes como microbios rotulados en experimentos relativos a la tuberculosis de reinfección. El uso de estos bacilos estreptomicinorresistentes se basa en la capacidad para diferenciar microbios estreptomicinosusceptibles y estreptomicinorresistentes en medios de cultivo. Los susceptibles no proliferan más que en medios sin estreptomicina, en tanto que los resistentes pueden proliferar tanto en los medios exentos como en los que contienen estreptomicina.

Si se ejecuta la inoculación inicial con bacilos tuberculosos virulentos, los microbios reinoculados subcutáneamente son detenidos en el sitio de la reinoculación y los ganglios linfáticos de drenaje. Sólo tratándose de dosis muy grandes de bacilos reinoculados es posible descubrir microorganismos en órganos remotos.

La cepa BCG y bacilos tuberculosos muertos fijados en parafina de una cepa virulenta, aunque producen inmunidad semejante, lo hacen en proporción mucho meno que los microbios virulentos.

Cuando la reinoculación tiene lugar en los pulmones, los bacilos se diseminan por todo el cuerpo, aun usando dosis mucho más pequeñas.

RESUME

A propos des problèmes de la réinfection en tuberculose. Le bacille tuberculeux streptomycino-résistant peut-il être utilisé comme bacille marqué dans les expériences de réinoculation?

Les bacilles tuberculeux streptomycino-résistants peuvent être utilisés comme germes marqués dans l'expérimentation de la tuberculose de réinfection. L'usage de ces bacilles tuberculeux streptomycino-résistants est fondé sur la possibilité de distinguer les germes streptomycino-sensibles des germes streptomycino-résistants dans les milieux de culture. Les germes streptomycino-sensibles poussent seulement sur les milieux dépourvus de streptomycine tandis que les germes résistants peuvent pousser à la fois sur les milieux renfermant ou non de la streptomycine.

Si l'inoculation initiale est effectuée avec des bacilles tuberculeux virulents, les germes réinoculés dans le derme sont arrêtés au point de réinoculation et dans les ganglions lymphatiques dépendant de ce territoire. Ce n'est que dans les cas où les doses de bacilles réinoculés

sont très élevées qu'il est possible de démontrer la présence du germe dans les organes éloignés.

La souche BCG et les bacilles tuberculeux tués d'une souche virulente introduits dans la paraffine, bien que produisant un effet d'immunité similaire ne le produisent pas à un degré aussi élevé que les germes virulents.

Quand le réinoculation s'effectue dans les poumons, les bacilles sont disséminés dans tout l'organisme, même quand des doses plus faibles sont utilisées.

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Case Report

THE USE OF TUBERCULIN IN THE TREATMENT OF TUBERCULOUS MENINGITIS[1]

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INTRODUCTION

In 1890, Robert Koch described the reaction now known as the Koch phenomenon. Since then, tuberculin has been widely used for the diagnosis and treatment of all types of tuberculosis, although Virchow has pointed out that tuberculosis may be disseminated by its use. With the advent of antituberculous chemotherapy, a new era, in which tuberculin is given in conjunction with these drugs, has begun. A review of the literature reveals such treatment in pulmonary tuberculosis (1), tuberculosis of bones and joints (2), and in tuberculous meningitis. Because of the drugs, the prognosis of tuberculous meningitis has improved considerably. However, there are still many fatal cases and survival cases with residual neurologic abnormalities.

The outcome of the treatment of tuberculous meningitis is influenced by several factors, such as age, race, other foci of disease, promptness in starting treatment, and particularly the pathologic characteristics of the disease.

The necropsy findings constantly show thick exudate in which acid-fast bacilli are embedded, surrounding the nervous tissue or blocking the subarachnoid space (3). Many attempts have been made to prevent the formation of exudate or to dissolve it when present. Such measures as the use of corticotropin and cortisone have been reported by several writers and have lately been reviewed by Johnson (4). The results were encouraging. The use of intrathecal streptokinase for the same purpose has not been promising (5).

The first published report on the use of intrathecal tuberculin in conjunction with streptomycin was made from England in 1950 by Smith and Vollum (3). They noted a very dramatic response in patients with advanced cerebrospinal block and severe neurologic damage, and believed this action to be specific because it appeared only in sensitized subjects. It was postulated that the acute inflammatory reaction as provoked by tuberculin activated a fibrinolytic process and brought the chemotherapeutic agents into contact with the site of the infection. On the other hand, Fletcher (6) showed through clinical, pathologic, and experimental evidence that intrathecal tuberculin, instead of causing any lysis of exudate, might actually contribute to its formation.

Swithinbank (7) concludes that the disturbance which follows the intrathecal injection of tuberculin is an antibody-antigen reaction. In order to provoke a meningeal reaction, purified protein derivative (PPD) must be delivered into the subarachnoid space. No meningeal reaction accompanies the clinical disturbance which follows the systemic administration of tuberculin.

Other reports by European writers appear in the literature (8, 9). Thus far, none has appeared in the United States. The largest series is that of Smith and associates, who in 1953 (10) collected thirty-seven cases treated with streptomycin and intrathecal

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tuberculin. The survival rate was significantly higher than in a similar series of intermediate and late cases treated with streptomycin alone.

CASE REPORT

D. H., thirty-nine years old, a white female, was admitted to the Boston Sanatorium for the second time on July 8, 1955. Her illness dates back to 1949 when she first developed gradual weakness of the legs which made walking impossible. While treated as an outpatient in two general hospitals, her symptoms cleared in four weeks. She felt well for more than three years, but was admitted to Boston City Hospital on August 18, 1953, with complaints of anorexia, weight loss, and nonproductive cough of several months' duration. A diagnosis of miliary tuberculosis with probable meningeal involvements was made on the basis of the following findings:

There was a positive family history for pulmonary tuberculosis. A roentgenogram of the chest showed mottled densities throughout both lung fields. Three sputum smears were positive for acid-fast bacilli. Biopsy of a left posterior cervical node showed acid-fast bacilli on microscopy. Lumbar puncture was not diagnostic. The electroencephalogram showed borderline T waves. Neurologic consultation supported the impression that the patient had tuberculous meningitis with a right cerebellar tuberculoma.

The patient was started on daily streptomycin, 1 gm.; para-aminosalicylic acid (PAS), 12 gm.; and isoniazid, 300 mg. the first week and 400 mg. thereafter. She was transferred to this institution on August 26, 1953, and was continued on the same regimen for three weeks, after which time streptomycin was reduced to 1 gm. twice a week.

While in this hospital, the patient had two convulsive seizures, thirty and forty days after admission. She was treated symptomatically with diphenylhydantoin (Dilantin®) and phenobarbital. Clinically, there was improvement consistent with a moderate regression of the infiltration on serial chest films. The patient left the hospital against medical advice seven months after admission.

During the following fifteen-month interval at home, neither follow-up observations nor treatment were carried out. On readmission to this hospital, the patient gave a history of two months' progressive weakness, loss of appetite, weight loss of 20 pounds, episodes of chills and night sweats. She denied neurologic symptoms. The temperature was 101°F., the pulse 120 per minute, her weight 92 pounds, and her blood pressure 90 systolic, 60 diastolic in mm. of mercury. Physical examination was within normal limits. Admission films showed increased infiltration and small cavities in both lungs, especially in the upper lobes. All sputum specimens were negative on microscopy but positive for tubercle bacilli on culture after eight weeks.

The patient was started on dihydrostreptomycin (1 gm. twice a week), PAS (12 gm.), and isoniazid (300 mg. daily). Ten days later, she had two convulsive seizures, two hours apart, preceded by sensations in the abdomen and relieved by sodium phenobarbital. The following day she had a similar seizure with clonic convulsions on the right. There was no loss of consciousness, tongue biting, or incontinence. The patient also complained of right-sided numbness, especially over the leg.

She was alert but nervous. Her sensorium was undisturbed and the pupils and ocular fundi were normal. No nystagmus was present. Sensory reaction to pain, pressure, heat and cold, and deep sensitivity also were normal. There was muscle weakness of the right arm as compared with the left. The finger-to-nose and heel-to-knee tests were positive on the right side. The deep tendon reflexes were normal. The patient was started on diphenylhydantoin, 100 mg. three times daily. On July 29 and 30, she had two seizures. Lumbar puncture on August 3 revealed a pressure of 120 mm. The cerebrospinal fluid was clear. The protein was 21 mg. per 100 ml. There were 12 cells per cu. mm., predominantly lymphocytes.

On August 14, thirty-six days after admission, the patient had a seventh convulsive

seizure. She attributed the convulsions to the antimicrobial drugs and discontinued isoniazid and PAS for approximately one week. Another lumbar puncture on August 22 showed no evidence of block, and the cerebrospinal fluid was clear. There were 22 cells per cu. mm., 95 lymphocytes; the chlorides were 763 mg. per 100 ml.; sugar, 52 mg. per 100 ml.; protein, 18 mg. per 100 ml. The colloidal gold reaction curve was 1112211000. On September 2, dihydrostreptomycin was increased to 1 gm. daily, isoniazid to 400 mg. daily, and phenobarbital (30 mg. three times daily) was added. Skull plates were reported normal. An electroencephalogram on September 16 showed a diffusely abnormal pattern, due to symmetrical slowing in the theta range. About this time the patient began to complain of profuse night sweats.

On September 21 (seventy-four days after admission) the patient's general condition deteriorated. She developed nausea and projectile vomiting, accompanied by headache, blurred vision, diplopia, and vertigo. She also became confused and disoriented. The neurologic findings were characterized by horizontal nystagmus and marked increase of all deep tendon reflexes. Lumbar puncture on September 26 revealed a xanthochromic cerebrospinal fluid obtained by aspiration. The Queckenstedt test was positive. The Pandy test was also positive. There were 6 cells per cu. mm., mostly lymphocytes. The smears contained tubercle bacilli and were confirmed by culture. Iproniazid phosphate (Marsilid®), 600 mg. daily, was given instead of isoniazid. Hydrocortisone, 100 mg. in dextrose solution, was administered intravenously on September 27 and was repeated daily for three days. A very short period of clinical improvement followed, when the patient did not vomit, but still complained of dizziness, numbness of the left foot, and blurring of vision. On October 1 and 2, the patient received 75 mg. of hydrocortisone intravenously, but her condition continued to deteriorate. She was semicomatose, there was bilateral Babinski, and the abdominal reflexes were absent.

Tuberculin Therapy

On October 3, lumbar puncture again yielded a positive Queckenstedt test and cloudy fluid negative for acid-fast bacilli. Intrathecal PPD, 0.00375 mg., was now started. The same day the patient was given cortisone, 80 mg. perorally. The following morning her temperature rose to 101°F. She was much more alert (able to respond to questioning and complaining of headache). Her fluid intake improved, and intravenous fluids were discontinued. The cerebrospinal fluid this time flowed spontaneously, was much clearer, and the Queckenstedt test became negative. There was a cell count of 422 per cu. mm., with 72 per cent granulocytes and 28 per cent lymphocytes. The protein was 63 mg. per 100 ml.; the sugar, 85 mg. per 100 ml.; and the chlorides, 621 mg. per 100 ml. The same dose of PPD was given but the cortisone was reduced to 70 mg. daily. The temperature dropped to 99°F. on October 5. The third dose of PPD was increased to 0.005 mg. and was not followed by fever. The cortisone was now reduced to 50 mg. in twenty-four hours. On October 7, the same dose of PPD was administered, without fever or other reaction. The sixth and seventh doses of PPD, therefore, were increased to 0.01125 mg. and were followed by moderate fever. Cortisone was reduced to 40 mg. on October 9, to 30 mg. on October 13, to 15 mg. on October 18, to 10 mg. on October 28, and was entirely omitted on October 31. The iproniazid had been reduced to 400 mg. daily on October 18. Drug-susceptibility studies in September revealed the tubercle bacilli in the sputum to be resistant to streptomycin alone, but susceptible to isoniazid and to isoniazid in combination with streptomycin.

Slight jaundice of the skin and conjunctivae were noticed for three weeks in October. Blood studies showed hypochromic and microcytic anemia, with a hemoglobin of 8.5 mg. per 100 ml. The urine was negative for bile. The bromsulphalein test showed 40 per cent retention after forty-five minutes. The erythrocyte fragility test was 0.28 per cent. After treatment, blood examination revealed a hemoglobin of 12 mg. per 100 ml.

Lumbar puncture on October 21 showed an initial pressure of 80 mm. of water; the cerebrospinal fluid was clear with 5 cells per cu. mm. mostly lymphocytes. The protein

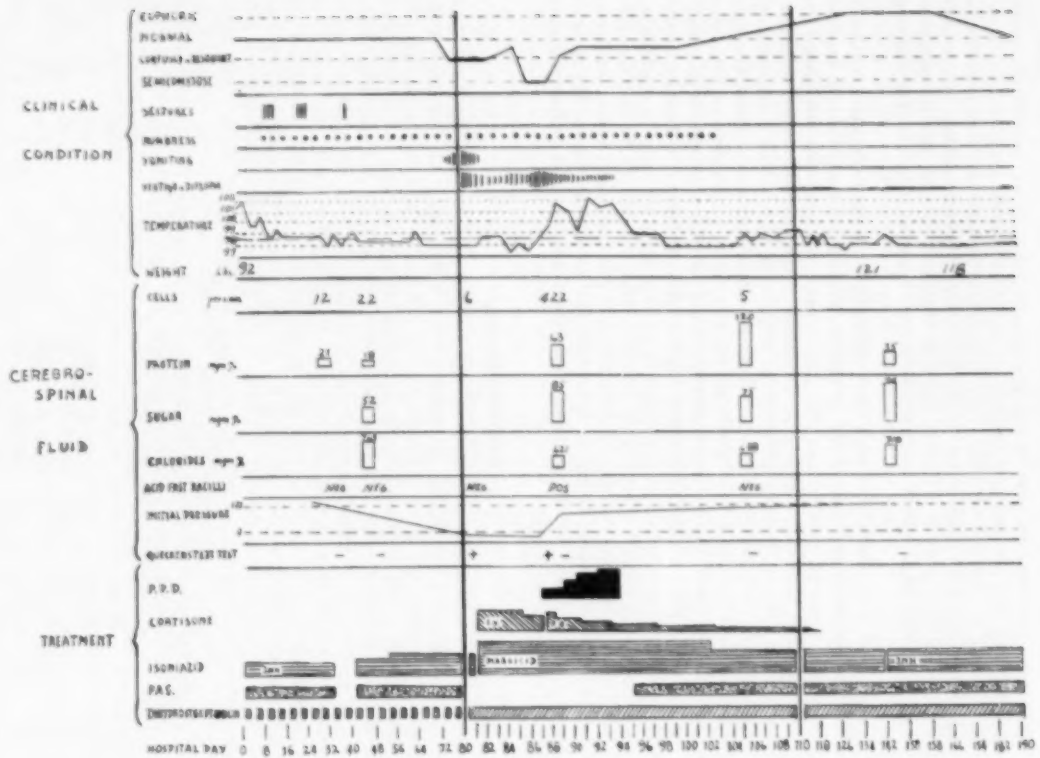

FIG. 1. Schematic representation of clinical course of *Patient D. H.*

was 120 mg.; sugar, 75 mg.; chlorides, 638 mg. per 100 ml. Smear and culture were negative for tubercle bacilli. The patient showed slow but steady clinical improvement. She had had a period of euphoria and enormous appetite, but was symptom free with no neurologic findings. She has gained thirty pounds and is ambulatory. On November 29, her cerebrospinal fluid was clear with an initial pressure of 100 mm. of water. The Queckenstedt test was negative. The protein was 35 mg. per 100 ml.; sugar, 94 mg. per 100 ml; and the chlorides, 710 mg. per 100 ml. Her chest film showed slight regression of the infiltration in both lung fields. Iproniazid was discontinued on November 29, and isoniazid, 400 mg. daily, was reinstituted. Streptomycin, PAS, phenobarbital, and polyvitamins are being continued (figure 1).

COMMENT

This patient was receiving routine therapy for pulmonary tuberculosis when she developed signs and symptoms of involvement of the central nervous system. Neurologic studies suggested tuberculoma of the left precentral area and the right cerebellar hemisphere with tuberculous meningitis. Despite intensification of her chemotherapy, the patient suddenly revealed evidence of more definite meningeal involvement, suggesting rupture of a tuberculoma into the meninges. At this time, and only then, the cerebrospinal fluid became positive for tubercle bacilli, and a cerebrospinal block became evident.

The encouraging response of patients with tuberculous meningitis and cerebrospinal block to corticotropin and corticosteroids in the hands of several investigators prompted the use of such an agent. When the patient got worse after six days on this therapy, however, intrathecal tuberculin was contemplated. Since, with respect to inflammation, the action of the corticosteroids was considered to be antagonistic to the action of tuberculin, it was decided to reduce the dose of hydrocortisone before the start of intrathecal tuberculin therapy. Because sudden withdrawal of cortisone might bring about adrenal insufficiency in the presence of stress, cortisone in decreasing doses was administered for four weeks and was then omitted.

When tuberculoprotein was first administered, there was definite evidence of cerebrospinal block. Puncture on the following day revealed no block; the fluid was cloudy with a high cell content. Never again was there evidence of block. There was also dramatic improvement of the patient's general clinical condition. The fever peaks were coincident with the increasing doses of tuberculin. At no time were there any signs of intracranial hypertension, a usual and sometimes dangerous complication of this therapy (3). After the seventh dose, the use of tuberculin was obviously justified. It accomplished its purpose on the basis of the clinical improvement and removal of the cerebrospinal block. Iproniazid was substituted for isoniazid because of the rapid deterioration of the patient's condition, and the latter drug was reinstituted because of the development of a marked euphoria.

This case illustrates that no routine schedule can be applied to the treatment of tuberculous meningitis. Thus far the follow-up is too short to permit definite conclusions. However, the results in the present case, together with reports from the literature, would indicate that intrathecal tuberculin has a place when conventional antituberculous chemotherapy appears to be failing or when there is evidence of cerebrospinal block or severe neurologic damage.

The danger of the use of tuberculin is quite evident, and the utmost caution must be observed. The regimen suggested by Smith and her group (3), in which the dosage is regulated according to the intradermal tuberculin test, is the safest to follow. Initially 0.1 ml. of Old Tuberculin, 1:1,000, is injected intradermally. PPD solution containing 0.0075 mg. per ml. is further diluted tenfold, a hundredfold, and eventually a thousandfold. If the skin test to tuberculin is 3-plus, 0.5 ml. of the thousandfold dilution is injected

intrathecally. The amount is doubled every other day according to reaction. If the skin test is 1-plus, 1 ml. of the thousandfold dilution is injected. All doses are tripled or quadrupled according to reaction.

It is impossible to determine on the basis of this case whether cortisone had any influence upon the dramatic response of the patient or the favorable end result. At any rate there is no reason to believe that cortisone was responsible for the removal of the cerebrospinal block. The restoration of normal cerebrospinal dynamics must be attributed to the tuberculin therapy.

SUMMARY

The use of intrathecal tuberculin in tuberculous meningitis is described. Reference is made to the implication of the pathologic characteristics of this disease. A case is reported which illustrates the beneficial results of intrathecal tuberculin in the presence of cerebrospinal block.

SUMARIO

El Uso de la Tuberculina en el Tratamiento de la Meningitis Tuberculosa con Presentación de un Caso

Describese el uso intratecal de la tuberculina en la meningitis tuberculosa. Se menciona la connotación de las características patológicas de esta dolencia. Discútese con minuciosidad una historia clínica que ilustra los resultados beneficiosos de la tuberculina por vía tecal cuando existe bloqueo raquídeo.

RESUME

Usage de la tuberculine dans le traitement de la méningite tuberculeuse. Observation d'un cas

L'article décrit l'emploi de la tuberculine intrathécale dans la méningite tuberculeuse. Les implications des caractéristiques pathologiques de cette affection sont mentionnées. L'observation d'un cas illustrant les résultats favorables de la tuberculine intrathécale en présence d'un blocage du liquide céphalo-rachidien est commentée en détail.

Acknowledgment

The writers would like to express their appreciation to Dr. David S. Sherman, Superintendent and Medical Director, Boston Sanatorium, and Dr. Heinz J. Lorge, Superintendent, Worcester County Sanatorium, for their assistance in the preparation of this paper.

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Notes

THE USE OF ZEPHIRAN[1] IN THE ISOLATION OF M. TUBERCULOSIS

A very large number of different media has been developed in an effort to achieve growth of *Mycobacterium tuberculosis* from the smallest number of viable organisms. Because of the relatively long time required for *M. tuberculosis* to grow into recognizable colonies, contamination has been a major problem. Abbott,[2] estimated that only two-thirds of the possible infectious cases in the field are detected by commonly used diagnostic methods. Control of contamination in these procedures has depended upon the bactericidal action of the sputum digestant coupled with the bacteriostatic action of the culture media.

Abbott,[3] incorporated penicillin in the digestive process, and Smith and associates[4] and Roberts and co-workers[5] employed penicillin in the culture media to reduce contamination. Freedlander,[6] Drea,[7] Smith and associates,[8] and Hirsch[9] have observed that tubercle bacilli are readily susceptible to the bacteriostatic action of Zephiran, but are remarkably resistant to its bactericidal action. The results of the present investigation show that Zephiran, when incorporated with trisodium phosphate as a digestant, will, under field conditions, significantly reduce contamination as well as increase the number of positive isolations as compared with standard procedures.

Petragnani medium as modified by Frobisher[10] was employed in all culture studies. Sputum specimens were digested either by the sodium hydroxide-hydrochloric acid procedure of Petroff[11] or by the trisodium phosphate concentration method of Corper and Stoner.[12] Growth on solid medium was determined by visual observation and was recorded in plus values using a control as 4-plus and comparing growth on experimental tubes with that of the control.

Bactericidal effect of Zephiran: To determine the viability of *Mycobacterium tuberculosis* var. *hominis* in Zephiran, cells from a stock culture of a virulent organism were suspended in 5 ml. of a 1:1,000 dilution of Zephiran. After four hours, a loopful of the suspension was cultured on Petragnani medium. To determine if the "carry-over" of Zephiran was inhibitory, the remainder of the suspension was centrifuged at 1,800 to 2,000 r.p.m. for thirty minutes. The supernatant was discarded and 5 ml. of saline were added. After resuspending the bacilli, a loopful was inoculated on another Petragnani slant. Following this washing, the organisms were re-centrifuged and the sediment was re-suspended in 5

[1] Zephiran is the proprietary name of a mixture of high molecular, alkyl-dimethyl-benzyl-ammonium chlorides manufactured by Winthrop-Stearns, Inc.

[2] Abbott, J. N.: Am. J. Pub. Health, 1950, *40*, 833.

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TABLE 1

VIABILITY OF M. TUBERCULOSIS SUSPENDED IN ZEPHIRAN

Time Exposure to 1:1,000 Zephiran	Growth (No Saline Rinse)	Growth (One Saline Rinse)
Saline control	4+	4+
4 hours	4+	4+
1 day	4+	4+
2 days	3+	4+
3 days	4+	4+
4 days	4+	4+
5 days	4+	3+
6 days	3+	4+
7 days	3+	4+
8 days	3+	4+
9 days	3+	3+
10 days	3+	3+
11 days	2+	3+

ml. of a 1:1,000 solution of Zephiran. This process was repeated at twenty-four-hour intervals for eleven days.

Examination of table 1 shows that the cells were still viable after being in contact with Zephiran for eleven days. It should be noted, also, that the carry-over of Zephiran did not significantly inhibit the growth on Petragnani medium.

Bactericidal effect of Zephiran in the presence of trisodium phosphate: Trisodium phosphate is one of the common sputum digestants used in the clinical laboratory. If Zephiran proved nontoxic for tubercle bacilli when incorporated with the digestant, it might prove useful in further reducing contamination.

Two series of ten tubes containing 10 per cent trisodium phosphate and dilutions of Zephiran ranging from 1:250 to 1:32,000 were set up. The control tube contained only saline. The same stock strain of *M. tuberculosis* used in the previous experiment was inoculated into each of the ten tubes of both series, and allowed to stand two hours at room temperature. One series of tubes was neutralized with hydrochloric acid, using brom thymol blue as an indicator. The other series was not neutralized. All tubes were centrifuged for thirty minutes at 2,000 r.p.m. and the sediment was suspended in saline and re-centrifuged. One loopful of sediment from each tube was streaked on a tube of Petragnani medium and incubated at 37.5°C.

Zephiran in concentrations from 1:250 to 1:32,000 in combination with trisodium phosphate did not prove bactericidal to *M. tuberculosis* var. *hominis* after two hours' exposure.

Comparison of trisodium phosphate-Zephiran digestion with the sodium hydroxide method on the viability of H37Rv:[13] The scrapings from an entire slant of H37Rv, grown on Petragnani medium, was emulsified in 50 ml. of sterile saline. Four rows of sterile screw-capped tubes were set up. Tubes in the first row contained 1.0 ml. of 4 per cent sodium hydroxide plus one drop of brom thymol blue, 0.5 ml. of sterile saline, and 0.5 ml. of bacterial suspension per tube. Each tube in the second and third rows contained 1.0 ml. of 10 per cent trisodium phosphate, 0.5 ml. of sterile saline, 0.5 ml. of bacterial suspension, and sufficient Zephiran to give a final concentration of 1:1,000. Each tube in the third row was centrifuged

[13] Obtained from the Department of Bacteriology, University of Nebraska.

TABLE 2

COMPARISON OF DIGESTION METHODS ON VIABILITY OF M. TUBERCULOSIS

Contact Time	Sodium Hydroxide	Trisodium Phosphate-Zephiran (Unwashed)	Trisodium Phosphate-Zephiran (One Washing)	Saline Control
0	4+	4+	4+	4+
1 hour	3+	4+	4+	4+
2 hours	3+	4+	3+	4+
3 hours	3+	4+	3+	4+
4 hours	3+	4+	4+	4+
5 hours	3+	4+	3+	4+
6 hours	3+	2+	4+	4+
7 hours	2+	3+	3+	4+
1 day	1+	3+	4+	4+
2 days	2+	4+	4+	4+
3 days	1+	2+	4+	4+
4 days	—	2+	3+	4+
5 days	—	—	3+	4+
6 days	—	1+	1+	3+
7 days	—	1+	—	3+
8 days	—	—	—	2+

and the cells re-suspended in saline prior to incubation. Tubes in the fourth row contained 1.5 ml. of sterile saline and 0.5 ml. of bacterial suspension. Standard loopfuls of the suspensions were removed and inoculated directly on Petragnani medium after each period of exposure.

The results tabulated in table 2 show that trisodium phosphate plus Zephiran was less toxic than sodium hydroxide. Viable organisms could be obtained after exposure to trisodium phosphate-Zephiran for seven days, whereas three days was the maximal survival time in sodium hydroxide.

Trisodium phosphate-Zephiran digestion of clinical specimens: Preliminary experiments using pure cultures can only indicate the potential usefulness of an isolation procedure. Therefore, the trisodium phosphate-Zephiran method of treating sputum was compared with the commonly used sodium hydroxide method in the isolation of mycobacteria from clinical specimens.

Ninety-five sputum samples were obtained from the State Tuberculosis Hospital at Kearney, Nebraska. Many patients from whom the specimens were obtained were under treatment, which increased the possibility of obtaining specimens positive for tubercle bacilli on microscopy but negative on culture. The samples were approximately twenty-four hours old when received at the laboratory.

Five milliliters of each sputum sample were added to two 50 ml. glass-stoppered centrifuge tubes. To one tube an equal quantity of 4 per cent sodium hydroxide was added, and to the other tube an equal quantity of 10 per cent trisodium phosphate-1:1,000 Zephiran mixture. During the fifteen minute digestion process, the specimens were shaken by hand periodically. Both sets of specimens were then centrifuged at 3,000 r.p.m. for fifteen minutes. The supernatant was poured off both sets. The sediment in the sodium hydroxide tubes was neutralized and two standard loopfuls of the sediment were inoculated on Petragnani medium. Approximately 48 ml. of sterile saline were added to the sediment of the trisodium phosphate-Zephiran tubes. These were vigorously shaken and re-centrifuged, and two stand-

TABLE 3

COMPARISON OF SODIUM HYDROXIDE AND TRISODIUM PHOSPHATE-ZEPHIRAN DIGESTION ON DETECTION OF M. TUBERCULOSIS IN 95 SPUTUM SAMPLES

Digestion Method	Positive Smears	Positive Cultures	Contaminated Cultures
	(per cent)	*(per cent)*	*(per cent)*
Sodium hydroxide	69.5	50.5	13.7
Trisodium phosphate-Zephiran	67.3	60.0	2.1

TABLE 4

COMPARISON OF TRISODIUM PHOSPHATE AND TRISODIUM PHOSPHATE-ZEPHIRAN DIGESTION ON DETECTION OF M. TUBERCULOSIS IN 153 SPUTUM SAMPLES

Digestion Method	Positive Smears	Positive Cultures	Contaminated Cultures
	(per cent)	*(per cent)*	*(per cent)*
Trisodium phosphate	17	21.6	7.2
Trisodium phosphate-Zephiran	17	32.7	0.7

ard loopfuls of the sediment were cultured. Smears were prepared from each of the digested sediments and stained by the Ziehl-Neelsen technique.

The results are tabulated in table 3. Two and two-tenths per cent more smears positive for tubercle bacilli were obtained from samples digested with sodium hydroxide. However, 9.5 per cent more positive cultures were obtained from samples digested with trisodium phosphate-Zephiran. Contamination was reduced by 11.6 per cent in samples digested with trisodium phosphate-Zephiran.

Additional clinical specimens were obtained from the Veterans Hospital in Lincoln, Nebraska. These specimens were from untreated patients, and included many in which the search for *M. tuberculosis* was requested as a routine procedure to exclude tuberculosis.

One half of each sputum sample was treated in the routine manner with trisodium phosphate. The other half was treated with trisodium phosphate-Zephiran. The sediments, after one washing, were streaked on Petragnani medium, and smears were made and stained by the Ziehl-Neelsen method.

Examination of table 4 shows that trisodium phosphate-Zephiran digestion resulted in 11.1 per cent more positive cultures than the routine trisodium phosphate method. In addition, 6.5 per cent more of the cultures were contaminated after treatment with trisodium phosphate alone.

* * *

The results of this investigation confirm the findings of Freedlander[6] and Hirsch[9] that bactericidal action of Zephiran on the tubercle bacillus is low.

Incorporation of 1:1,000 Zephiran with trisodium phosphate in the digestion of sputum samples showed a marked improvement in the elimination of contaminants and, thus, greater success in obtaining positive cultures for *M. tuberculosis*. In this respect, this procedure is also superior to the penicillin-incorporation method.

Aside from being a useful incorporant in the digestive process, Zephiran, because of its low bactericidal action on the tubercle bacillus, might be useful in reducing the growth of contaminants which occurs during transit of sputum samples. In many instances,

sputum samples are collected from out-of-state patients, and several days elapse while the specimen is en route to the diagnostic laboratory. During this interval, contaminants multiply and the efficiency of the isolation procedure is decreased. It is quite possible that Zephiran could be used in sputum containers which are mailed to outpatients, thus reducing growth of contaminants during transit.

In summary: M. tuberculosis remains viable in a 1:1,000 dilution of Zephiran for eleven days. Positive cultures of *M. tuberculosis* were obtained after exposure to 1:1,000 Zephiran in 10 per cent trisodium phosphate for six days. Incorporation of Zephiran with trisodium phosphate in the digestion of sputum is superior to either of the digestive methods in routine use for isolation of *M. tuberculosis*. This method gave 9.5 per cent and 11.1 per cent more positive cultures than the standard sodium hydroxide and trisodium phosphate methods, respectively.

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THE OCCURRENCE OF HYPERURICEMIA DURING PYRAZINAMIDE-ISONIAZID THERAPY

Since the fifty-bed tuberculosis unit of Albany Veterans Hospital was activated in 1952, 3 patients developed clinical gout, all after receiving antituberculous drugs for many months. When the third case occurred in January, 1956, the present writers became suspicious that this unusual frequency of gout might be related to the treatment which the patients were receiving.

The first case, involving the second toe, occurred in December, 1954, in a patient who was receiving streptomycin twice weekly and isoniazid each day. He had a history of previous attacks of gout. One of the other 2 patients developed gout in the great toe in December, 1955; the other, in the tendon of the tibialis posterior in January, 1956, after receiving pyrazinamide (3 gm.) and isoniazid (300 mg.) daily, for thirteen and for eight months, respectively. Although neither of these patients ever had had an attack of gout before, the most recently affected patient had a family history of gout. In all 3 of these patients the gout responded within a few hours to colchicine administration. No further attacks have occurred and the antituberculous drugs received by the patients have been continued without any change.

In order to detect any relationship of the antituberculous drugs to gout, fasting serum uric acid concentrations were determined in a few patients, selected at random, on all of the major drug regimens. The blood was drawn in the morning approximately sixteen hours after the last dose of medication. The serum uric acid was determined by the modification of the method of Kern and Stransky, described in the 1950 edition of Fister's *Manual of Standardized Procedures for Spectrophotometric Chemistry*. In this laboratory the normal range of values for the serum uric acid is 2.8 to 5.5 mg. per 100 ml. of serum. All uric acid determinations performed since January, 1956, are noted in table 1.

The day-to-day fluctuations of the serum uric acid concentrations are evident. Patients *DeL* and *McC* are the ones who developed clinical gout. All of the patients tested had been on antituberculous drugs for many months except the patient *Pem*, on whom uric acid determinations before and after initiation of treatment were obtained, and patients *Sie* and *Fis* who were undergoing therapy for only a few weeks.

All of these patients had normal hepatic and renal function and normal blood counts. In the first case of gout which occurred in December, 1954, the serum uric acid was 8.4 mg. per 100 ml. This case is not entered in table 1. The cases are listed in table 1 under the basic chemotherapy regimens received, and any additions or deletions of pyrazinamide are noted under *Remarks*. The final value obtained as a result of these changes is entered in the appropriate column. Only men were available for study.

As may be seen in table 1, the serum uric acid reached concentrations well above normal in all patients who received pyrazinamide (3 gm.) and isoniazid (300 mg.) daily except patient *Sie*. In this case the uric acid rose only to 5.7 mg. per 100 ml. five days after pyrazinamide was added to the isoniazid–para-aminosalicylic acid (PAS) regimen. This hyperuricemia occurred in both of the patients who never manifested clinical gout as well as in the 2 who did, and in the latter it persisted after the acute attack had subsided.

Patients who received 3 gm. of pyrazinamide with drugs other than isoniazid had uric acid values at or slightly above the 6 mg. upper limit of normal (*Hyn*, *Ran*, *McB*, and *Gan*). When the pyrazinamide was increased to 6 gm. daily (*McB* and *Gan*) and continued for another three days, the uric acid rose almost a milligram higher to values of

TABLE 1

URIC ACID CONCENTRATIONS IN BLOOD AND URINE

Patient	Serum Uric Acid			24-Hour Urine Output		Remarks
	Before Pyrazinamide	During Pyrazinamide	Off Pyrazinamide	During Pyrazinamide	No Pyrazinamide	
Patients Receiving Pyrazinamide and Isoniazid						
	mg. per 100 ml.			*mg.*		
Hal		9.4, 9.2		333		Uric acid fasting = 9.2; 2 hours after pyrazinamide-isoniazid = 9.5
Dew		8.9, 9.5		388		Uric acid fasting = 9.5; 2 hours after pyrazinamide-isoniazid = 10.1
Gra		6.9, 8.7		570		
Pro		10.1, 8.2	3.4			Pyrazinamide stopped; one day uric acid = 7.0; 5 days = 3.4
Pem	4.3	8.9		315	644	One day of pyrazinamide, uric acid = 5.9; 2 days = 7.2
DeL		9.6, 6.1, 8.7	4.7	280		Gout December, 1955, pyrazinamide stopped, one day uric acid = 6.8; 2 days = 5.5
McC		14.4, 9.8, 11.1	6.1	374	917	Gout January, 1956, on Benemid urine = 560, pyrazinamide stopped; 3 days uric acid = 6.1; 4 days = 6.1
Patients Receiving Pyrazinamide and Drugs Other Than Isoniazid						
Hyn		5.4, 4.5				Streptomycin, PAS, pyrazinamide
Ran		5.1, 7.0				Viomycin, pyrazinamide
Patients Receiving PAS and Isoniazid						
Cau	3.4					
Hil	3.6					
VanR	2.8					
Scho	3.0					
Wha	3.8					
Sie	3.0	5.7				4 days of pyrazinamide, uric acid = 5.7; 5 days = 5.7
Patients Receiving Streptomycin-Isoniazid						
Con	5.1					
DeC	4.8					
Gri	3.9					
Car	5.1					
Cro	3.9					
Ros	5.0					
Fis	3.6	7.7				3 days of pyrazinamide, uric acid = 7.4; 4 days = 7.7
Patients Receiving Streptomycin-PAS						
Vro	4.8					
Fit	2.9					
MacP	3.0					
McB	2.8	6.1				3 days of pyrazinamide (3 gm.), uric acid = 6.1; 3 more days @ 6 gm. = 7.0
Gan	5.9	6.8				3 days of pyrazinamide (3 gm.), uric acid = 6.8; 3 more days @ 6 gm. = 7.6

7.0 and 7.6 mg. per 100 ml., respectively. These findings suggest that the pyrazinamide is the important factor in the production of hyperuricemia and that isoniazid enhances its effect.

The hyperuricemia became fully established three days after institution of pyrazinamide-isoniazid therapy (*Pem*), increasing from 4.3 to 8.9 mg. per 100 ml. This also occurred when pyrazinamide was given to a patient already receiving isoniazid with streptomycin (*Fis*), the uric acid increasing from 3.6 to 7.7 mg. per 100 ml. Another patient (*Sic*) who received isoniazid-PAS had a rise in uric acid from 3.0 to 5.7 mg. per 100 ml. four days after pyrazinamide was added. The values failed to rise any higher after another day of pyrazinamide. The relationship of PAS and its possible uricosuric effect to the failure of the uric acid to rise well above normal in this patient and others who received it needs further investigation.

When pyrazinamide alone was discontinued, the previously elevated serum uric acid fell to normal within three days in the 2 patients who had previously had clinical gout (*DeL* and *McC*) and within five days (no specimen being obtained from the second through fourth day) in one patient (*Pro*) who did not have any joint symptoms.

The serum uric acid did not change significantly two hours after the morning dose of pyrazinamide and isoniazid, the fasting state being maintained (*Hal* and *Dew*). The uric acid rose 0.3 mg. in the first instance and 0.6 mg. in the second.

The twenty-four-hour urinary output of uric acid varied but was in the low range of normal. The pretreatment output of uric acid in one patient (*Pem*) was 644 mg., while after being on pyrazinamide-isoniazid for one week the value fell to 315 mg. Another patient (*McC*) while on probenecid (Benemid®) to prevent recurrence of his gout excreted 560 mg. of uric acid. After the probenecid had been discontinued for a week the uric acid excretion fell to 374 mg. Then the pyrazinamide was stopped for three days and the output was 917 mg. in the next twenty-four hours.

It is interesting that in the 2 patients (*Pem* and *McC*) tested with and without pyrazinamide a decreased urinary uric acid output was noted, in spite of an elevated serum uric acid, while on pyrazinamide. This suggests the possibility of a renal effect. However, in view of the known daily fluctuation of uric acid output this preliminary finding needs confirmation.

As a chemical control on these observations, isoniazid was added to serum *in vitro*, producing a concentration of 10 γ per ml. and pyrazinamide was added to produce a serum concentration of 40 γ per ml. The two drugs were also added individually to specimens of the same serum. In no case did the addition of the drugs change the original untreated serum uric acid concentration of 4.1 mg. per 100 ml.

In the patients who received pyrazinamide-isoniazid there has been no clinical evidence of renal calculi or tophi. There have been no joint pains such as those described by Yeager and associates[1] in their original report on pyrazinamide alone and by Campagna and his associates[2] on its use with isoniazid. In the 2 cases of gout which did occur in the present study each patient had a swollen, hot, tender joint.

The clinical implications of the hyperuricemia which occurred on pyrazinamide-isoniazid therapy are interesting and may prove to be far reaching. Apparently it is not due to physical presence of these drugs in the serum, but is a delayed response requiring approximately two to three days to develop and disappear. Pyrazinamide in a daily dose of 3 gm. without isoniazid raises the serum uric acid to, or just above, the upper limit of normal and 6 gm. daily raises it another milligram per 100 ml. The possible relationship

[1] Yeager, R. L., *et al.*: Am. Rev. Tubere., 1952, *65*, 523.
[2] Campagna, M., *et al.*: Am. Rev. Tubere., 1954, *69*, 334.

of these findings to the metabolism of uric acid, primary and secondary gout, the tubercle bacillus, and the long-range effects of hyperuricemia on the patient receiving pyrazinamide-isoniazid therapy have yet to be developed.

Addendum

Since the above Note was submitted for publication, work in progress confirms the hyperuricemic effect of pyrazinamide. The results thus far still suggest that there is a decreased urinary output of uric acid during pyrazinamide therapy and that PAS diminishes the hyperuricemic effect of pyrazinamide and is probably uricosuric. The findings are inconclusive whether isoniazid further increases the hyperuricemia produced by pyrazinamide.

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FURTHER OBSERVATIONS ON THE PREVALENCE OF STREPTOMCYIN- AND ISONIAZID-RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS IN PATIENTS WITH NEWLY DISCOVERED AND UNTREATED ACTIVE PULMONARY TUBERCULOSIS[1]

In a previous publication,[2] the present writers reported on the prevalence of streptomycin-resistant and isoniazid-resistant strains of tubercle bacilli in the sputum of clinic patients with untreated[3] active pulmonary tuberculosis discovered during the interval between July, 1953, and January, 1955. It is the purpose of the present Note to report the observations made in a similar but more intensive study, which included hospital as well as clinic patients discovered to have active and untreated pulmonary tuberculosis in New York City during the year 1955.

Using the same methods for collection and laboratory investigation as were previously described,[2] sputum specimens were obtained from a large number of patients newly admitted to most of the tuberculosis services of the municipal hospitals of New York City and to all of the chest clinics of the Bureau of Tuberculosis during the study period (January 1, 1955, to December 31, 1955).

The present series of patients was selected, using essentially the same criteria that were described in the first report, from the very much larger group of patients who submitted sputum specimens. The present series, in contrast to the one already reported, was limited to patients with *newly reported* bacteriologically proved active pulmonary tuberculosis; patients with *recently reactivated* tuberculosis were excluded. In addition, for the purpose of the present study, the definition of a *newly reported case of active pulmonary tuberculosis* was modified to read as follows: A patient whose sputum specimens were received in the laboratory within a period of *three months* (previously six months) following the date when the diagnosis of active pulmonary tuberculosis was reported for the first time. These minor modifications for selection were made in order to limit the present series to a group of patients whose previous antituberculous drug therapy could be more accurately investigated than was possible in the first study.

* * *

Six hundred and thirty-one patients were finally included in this series. Of these, 466 patients originated from hospitals and 165 from clinics. An analysis of the age, race, sex, and stage of disease in this group of patients revealed a distribution strikingly similar to that of the entire group of newly diagnosed cases of active pulmonary tuberculosis in persons more than ten years of age registered in New York City during the year 1955. It is believed, therefore, that these 631 patients were a representative sample of the entire group from which they were selected.

The cultural findings of the sputum for the present series of patients are noted in table 1. Some growth of *M. tuberculosis* on drug-containing medium was observed in 76 instances, or 12 per cent (the corresponding figure in the 1953–1954 study was 11.2 per cent). The proportion of strains showing some growth on drug-containing medium varied from 8.7 per cent among the 241 strains with less than 1-plus growth in the control culture to 17.2 per cent among the 157 strains with 3-plus or 4-plus growth in the

[1] This study was supported, in part, through a contract (No. SAth 00795, March 30, 1954) with the Public Health Service.

[2] Chaves, A. D., *et al.*: Am. Rev. Tuberc., 1955, *72*, 143.

[3] For the purpose of this report, all references to treatment will apply to antimicrobial treatment (streptomycin or isoniazid).

TABLE 1

QUANTITATIVE CULTURAL FINDINGS ON DRUG-FREE AND DRUG-CONTAINING MEDIA OF SPUTUM FROM 631 UNTREATED NEWLY REPORTED ACTIVE CASES OF PULMONARY TUBERCULOSIS

Departments of Health and Hospitals, City of New York

January 1, 1955, to December 31, 1955

Growth on Drug-Containing Media	Total		Quantitative Findings on Drug-Free Medium (Control)					
			4+ and 3+		2+ and 1+		Less Than 1+	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	631	100.0	157	100.0	233	100.0	241	100.0
Hospital Cases	*466*		*134*		*186*		*146*	
Clinic Cases	*165*		*23*		*47*		*95*	
No growth on any drug-containing media	555	88.0	130	82.8	205	88.0	220	91.3
Some growth on drug-containing media	76	12.0	27	17.2	28	12.0	21	8.7
Streptomycin alone	35	5.5	13	8.3	12	5.2	10	4.1
Isoniazid alone	39	6.2	14	8.9	15	6.4	10	4.1
Both streptomycin and isoniazid	2	0.3	0		1	0.4	1	0.4

TABLE 2

DISTRIBUTION OF CULTURES POSITIVE FOR TUBERCLE BACILLI FROM 631 UNTREATED PATIENTS BY DEGREE OF RESISTANCE SHOWN TO STREPTOMYCIN AND ISONIAZID

Type of Case	Total Positive Cultures*	Streptomycin Resistance					Isoniazid Resistance				
		No Growth on Any Streptomycin-Containing Medium	Some Growth on Streptomycin-Containing Medium			Total	No Growth on Any Isoniazid-Containing Medium	Some Growth on Isoniazid-Containing Medium			Total
			Highest Concentration in γ per Ml. of Medium on Which Any Growth Was Seen					Highest Concentration in γ per Ml. of Medium on Which Any Growth Was Seen			
			10	100	1,000			0.1	1.0	5.0	
Hospital	466	438	24	1	3	28	435	28	0	3	31
Clinic	165	156	8	1	0	9	155	7	2	1	10
Total	631	594	32	2	3	37	590	35	2	4	41

* Includes 2 patient-strains which showed some degree of resistance to both streptomycin and isoniazid.

control culture. There was no appreciable difference in distribution among hospital and clinic cases that could not be explained on the basis of the difference in the distribution of the quantitative findings in the control culture. In general, growth of the tubercle bacilli was heavier in the cultures inoculated with specimens from hospital patients.

Of the 76 patient-strains showing any growth on drug-containing medium, 35 showed some growth on streptomycin-containing medium; 39, on isoniazid-containing medium; and only 2 showed some growth on both streptomycin- and isoniazid-containing media

TABLE 3

PROPORTION OF 631 UNTREATED PATIENTS WITH RECENTLY DISCOVERED ACTIVE TUBERCULOSIS WHOSE ORGANISMS SHOWED SIGNIFICANT RESISTANCE TO STREPTOMYCIN* OR ISONIAZID†

Drug to Which Significant Resistance Was Demonstrated‡	Quantitative Findings on Drug-Free Medium (Control)						Total (631 Patients)	
	4+ and 3+ (157 Patients)		2+ and 1+ (233 Patients)		Less Than 1+ (241 Patients)			
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Streptomycin	0	—	2	0.85	1	0.41	3	0.48
Isoniazid	1	0.64	3	1.30	1	0.41	5	0.80

* Significant resistance to streptomycin: any growth on 1,000.0 γ of streptomycin per ml. of medium or growth on 100.0 γ of streptomycin per ml. equivalent to that on the control culture.

† Significant resistance to isoniazid: any growth on 5.0 γ of isoniazid per ml. of medium or growth on 1.0 γ of isoniazid per ml. equivalent to that on the control culture.

‡ No patient had organisms which were significantly resistant to both streptomycin and isoniazid.

(*see* table 1). In table 2 the distribution is indicated of all the sputum specimens positive for tubercle bacilli, according to the degree of resistance shown to streptomycin and isoniazid as measured by the highest concentration of the drug which permitted any growth. Only 5 patient-strains showed *any* growth on media containing 100 γ or more of streptomycin per ml., and only 6 showed *any* growth on media containing 1.0 γ or more of isoniazid per ml.

The number and percentage of patients whose sputum contained strains of *M. tuberculosis* displaying significant resistance to streptomycin and isoniazid, as previously defined,[3] are shown in table 3. Among the 390 patient-strains with 1-plus to 4-plus growth in the control cultures, 2, or approximately 0.5 per cent, were significantly resistant to streptomycin and 4, or 1.0 per cent, were significantly resistant to isoniazid. In the earlier study, the corresponding figures for significant drug resistance were 1.5 per cent and 2.9 per cent for streptomycin and isoniazid, respectively.

Among the 76 patients with strains of tubercle bacilli which showed any growth on drug-containing medium, there were 6 with a history of exposure to a previously treated case of active tuberculosis. Further details concerning the possible source cases were not obtained.

* * *

For the sake of completeness, to supplement the present study in the adult population, the writers obtained information on the results of drug-susceptibility tests done at Bellevue Hospital on the specimens from children admitted for previously untreated tuberculosis to the Pediatric Service of that hospital during 1955.[4] Tubercle bacilli were isolated from 32 of the 88 such children admitted. All 32 of the patient-strains isolated were entirely susceptible both to streptomycin and isoniazid, using the indirect testing method.

Thus, a most intensive search for primary drug resistance in tubercle bacilli isolated from newly diagnosed cases of tuberculosis in New York City during 1955 revealed no significant change from the findings described in a more limited study conducted between July, 1953, and January, 1955. The smaller percentage of significantly drug-resistant

[4] Data supplied through the courtesy of Dr. Edith M. Lincoln.

strains in the 1955 study as compared with the one conducted in 1953–1954 is probably due to more careful screening in the present series. The previous conclusion that primary drug resistance in patients with tuberculosis does not at present constitute a serious public health problem is, therefore, corroborated. Studies, however, must be constantly carried out to detect, as early as possible, any unfavorable change that may occur in the present fortunate situation.

In summary: In New York City, during 1955, tubercle bacilli were isolated from a study population of 631 adults and 32 children with previously untreated tuberculosis. The patient-strains of tubercle bacilli from adults were tested for susceptibility to streptomycin and isoniazid by the direct method; the indirect method was used for the patient-strains from infants and children. In 555 adult patients (88 per cent) and in all of the pediatric patients the strains isolated were completely susceptible to both streptomycin and isoniazid. Of the 76 patients (12 per cent) from whom strains were isolated which showed any degree of resistance to either drug, only 3 (0.5 per cent of the total) had strains significantly resistant to streptomycin and only 5 (0.8 per cent of the total) had strains significantly resistant to isoniazid.

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INSTABILITY OF THE POTENCY OF TUBERCULIN DILUTIONS

A Second Report

It is generally assumed that tuberculin is unstable when highly diluted and that the weak dilutions used for intradermal testing, therefore, should be stored for a short time only. In extensive studies carried out to obtain more precise information on this point, the present writers stored tuberculin dilutions for periods ranging from a few days to many months. The results obtained did not support the assumption that the potency of the dilution decreases systematically with duration of storage. In fact, the studies failed to reveal any loss of potency for dilutions of 5 tuberculin units (TU) stored up to nine months at low temperature. The same studies, however, revealed unsystematic variations in potency between dilutions made at different times from the same product and also between ampoules filled with the same dilution.

In order to identify the source of this unexpected "inter-ampoule variation," a further series of studies was started. As reported previously,[1] these studies resulted in the discovery of a peculiar phenomenon—the "volume effect." It was found that, when an ampoule is filled only partially with a dilution of purified tuberculin, the potency of the dilution is substantially reduced within a day or even a few hours.

It is assumed that the two phenomena, the "inter-ampoule variation" and the "volume effect," may be due to a common cause. As the "volume effect" is more easily reproduced and measured, it was made the subject of further experiments.

The purpose of the present paper is to illustrate briefly certain new aspects of this phenomenon and indicate the nature of its cause.

In all experiments reported here, the potency of tuberculin was measured by intradermal testing in white male BCG-vaccinated guinea pigs.

Each animal was given from six to ten tests in the skin of the back or flanks, 0.1 ml. of the dilution being injected for each test. The reactions were measured in approximately twenty-four hours by a trained observer who had no knowledge of which dilution of tuberculin had been used for the separate tests. The data given below are all based on the average of the longitudinal and transverse diameters of induration.

The guinea pigs were of uniform weight and had all been sensitized with Danish BCG vaccine (Statens Seruminstitut, Copenhagen) one to two months before experimentation. As the level of sensitivity varied considerably from animal to animal, it was found profitable to use statistical designs with adjustments for these variations. The tests allocated to each animal were randomly distributed among the sites used for testing.

Unless otherwise stated, the tuberculin used for experimentation was RT XIX-XX-XXI (Statens Seruminstitut, Copenhagen), in 5 TU dilution (containing 0.0001 mg. per 0.1 ml.). The diluent employed was a phosphate buffered sodium chloride solution with, in some cases, chinosol as a preservative. Except in two experiments in which different types of containers were tested, the dilutions were stored in rubber-capped ampoules of brown Jena glass, 85 mm. in length (neck excluded) and 20 mm. in diameter, with a total capacity of approximately 23 ml. The ampoules, which were factory-new, were rinsed in tap water and distilled water and dry-sterilized prior to use.

Different tuberculin products: The first experiments in which the "volume effect" was detected were made with the purified tuberculin RT XIX-XX-XXI. This brought up the question whether the "volume effect" is found with this tuberculin only or if it is obtained with other products as well.

[1] Guld, J., *et al.*: Am. Rev. Tuberc., 1955, *72*, 126.

TABLE 1

MEAN SIZE OF REACTIONS (MM.)* IN BCG-VACCINATED GUINEA PIGS TO INTRADERMAL TESTS WITH DILUTIONS OF DIFFERENT TUBERCULIN PRODUCTS IN COMPLETELY AND PARTIALLY FILLED AMPOULES

Tuberculin Dilution			First Experiment†			Second Experiment‡		
Production Center	Preparation	Strength	Degree of Filling		Volume Effect (1)-(2)	Degree of Filling		Volume Effect (3)-(4)
			Full (1)	½ Full (2)		Full (3)	½ Full (4)	
		mg./0.1 ml.						
Statens Seruminstitut	RT VII	1/10,000	10.9	7.0	3.9	12.1	5.1	7.0
Statens Seruminstitut	RT XIX-XX-XXI	1/10,000	11.6	7.2	4.4	12.4	5.6	6.8
Statens Seruminstitut	RT XXII	1/15,000	11.7	4.1	7.6	10.9	4.5	6.4
Henry Phipps Institute	PPD-S	1/10,000	13.1	12.6	0.5	14.4	12.8	1.6
Henry Phipps Institute	SOTT-4	1/10,000	—	—	—	12.5	9.4	3.1
Weybridge Laboratory	Weybridge PPD—mammalian	3/40,000	10.4	8.5	1.9	10.9	8.5	2.4
Weybridge Laboratory	Weybridge PPD, freeze-dried	1/10,000	—	—	—	11.4	5.5	5.9
Institut Pasteur	P.T.	1/10,000	12.5	12.2	0.3	12.4	11.3	1.1
Institut Pasteur	I.P. 48	1/10,000	—	—	—	12.5	11.0	1.5
Statens Seruminstitut	Koch's Old Tuberculin, strength 1.7	1/34	12.1	11.4	0.7	12.6	12.5	0.1
Hampstead	Standard Old Tuberculin	1/20	—	—	—	13.1	12.4	0.7

* Each mean is based on 8 tests.

† The tuberculin dilutions were stored in the ampoules for two days.

‡ The tuberculin dilutions were stored in the ampoules for one day.

A number of well-known products were tested in two experiments, one including seven and the other eleven different products. From each product a dilution roughly corresponding in strength to a 5 TU dilution of RT XIX-XX-XXI was prepared and stored for one day in ampoules, some of which were filled completely and some partially. The diluent described above was used for all products. The products tested and the results of the experiments are shown in table 1.

The strongest "volume effect" consistently occurred with the RT preparations but the effect was also substantial for the Weybridge preparations as well as for SOTT-4. The latter was prepared twenty years ago with approximately the same technique as that used for preparing RT. For PPD-S (the international standard for purified protein derivative of mammalian tuberculin) and the Institut Pasteur tuberculins the effect was small, and it was minimal or nonexistent for Old Tuberculin (OT).

The stability of the two preparations of OT may be explained by their content of foreign proteins (*see* Stabilizing Agents below) derived from the broth medium used for the preparation. In regard to the differences observed between the different purified tuberculins an explanation cannot be given at present. However, one may assume either that the different methods of preparation modify the tuberculin molecules to a different degree or that, with the different purification methods, different stabilizing impurities are removed. Experiments are being carried out to determine the influence on the "volume

TABLE 2

MEAN SIZE OF REACTIONS (MM.)* IN BCG-VACCINATED GUINEA PIGS TO INTRADERMAL TESTS WITH 5 TU DILUTIONS CONTAINING GUM ARABIC, GELATINE, AND TWEEN 80 IN DIFFERENT CONCENTRATIONS IN COMPLETELY AND PARTIALLY FILLED AMPOULES†

Concentration of Additive	Gum Arabic			Gelatine			Tween 80		
	Full Ampoules (1)	1/10 Full Ampoules (2)	Volume Effect (1)–(2)	Full Ampoules (3)	1/10 Full Ampoules (4)	Volume Effect (3)–(4)	Full Ampoules (5)	1/10 Full Ampoules (6)	Volume Effect (5)–(6)
%									
0	10.7	4.0	6.7	10.6	4.1	6.5	10.4	4.8	5.6
0.001	—	—	—	9.8	6.2	3.6	10.5	7.8	2.7
0.010	10.1	5.0	5.1	10.5	8.2	2.3	12.1	11.7	0.4
0.100	10.2	5.0	5.2	10.6	9.7	0.9	12.2	10.9	1.3
1.000	10.5	8.5	2.0	11.2	10.7	0.5	12.3	12.4	−0.1
10.000	10.8	10.5	0.3	—	—	—	—	—	—

* Each mean is based on 6 tests.

† The tuberculin dilutions were stored in the ampoules for one day.

effect" of the different steps in the purification procedures, i.e., concentration, precipitation, dehydration, et cetera.

Stabilizing agents: It is known that other purified proteins, such as purified preparations of toxins, enzymes, and viruses, are unstable in high dilution and that this instability may be prevented by the addition of "stabilizing agents" to the diluent. Gelatine is perhaps the most commonly used. For the stabilization of tuberculin Gottschall and Bunney[2] have recommended a buffered diluent containing 0.1 per cent gum arabic.

The ability of some of these stabilizing agents to prevent or, at least, reduce the "volume effect" was examined. The results of some of the experiments with gelatine, gum arabic, and Tween® 80[3] are given in table 2. All three substances markedly reduced the "volume effect," but gelatine and Tween 80 were much more effective than gum arabic when used in equal amounts. In experiments not reported here, stabilization was also obtained with human albumin, peptone, and glycerin broth.

Clearly, these findings suggest a practical solution to the problem. However, careful examination of the possible antigenic and toxic properties of the different stabilizing agents would be needed before such a solution could be accepted.

Various containers: In two experiments, different types of containers were used for the tuberculin dilutions. These experiments were made partly in an attempt to find a practical solution, but mainly in the hope that they would furnish a clue to the factors responsible for the "volume effect."

Tubes of nylon and quartz, ampoules of Jena glass with and without silicone-coating, and Erlenmeyer flasks of Pyrex® glass were used. The different containers were filled with 5 TU dilution—completely or to one tenth of capacity. All containers except the Erlenmeyer flasks were kept lying flat. The results and a detailed description of the containers are given in table 3.

The experiment yielded no practical solution, as a "volume effect" was found for all the containers. A quantitative evaluation of the "volume effect" for the different materials

[2] GOTTSCHALL, R., AND BUNNEY, W. E., J. Immunol., 1938, *34*, 103.

[3] Used by London and Hudson to stabilize acid prostatic phosphatase, Arch. Biochem., 1953, *46*, 141.

TABLE 3

MEAN SIZE OF REACTIONS (MM.) IN BCG-VACCINATED GUINEA PIGS TO INTRADERMAL TESTS WITH 5 TU DILUTIONS STORED IN CONTAINERS OF VARIOUS MATERIALS, ACCORDING TO DEGREE OF FILLING OF CONTAINER

Container	First Experiment*						Second Experiment†					
	Size			Degree of Filling			Size			Degree of Filling		
	Approximate Volume	Height	Diameter	Full (1)	½₀ Full (2)	Volume Effect (1)–(2)	Approximate Volume	Height	Diameter	Full (3)	½₀ Full (4)	Volume Effect (3)–(4)
	ml.	*cm.*	*cm.*				*ml.*	*cm.*	*cm.*			
Erlenmeyer flask	500	Erlenmeyer shape		11.8	10.8	1.0	500	Erlenmeyer shape		12.2	9.3	2.9
Erlenmeyer flask	40			10.9	7.4	3.5	50			11.7	6.2	5.5
Nylon tube	40	8.0	3.3	10.6	5.3	5.3	40	8.0	3.3	10.8	5.4	5.4
Quartz tube	10	10.0	1.5	10.4	6.8	3.6	20	16.0	1.6	9.4	5.7	3.7
Silicone-coated Jena glass ampoule	20	8.5	2.0	10.0	3.7	6.3	20	8.5	2.0	10.0	5.1	4.9
Jena glass ampoule	20	8.5	2.0	11.4	4.5	6.9	20	8.5	2.0	11.4	5.4	6.0

* Each mean is based on 8 tests. The tuberculin dilution was stored in the containers for two days.

† Each mean is based on 12 tests. The tuberculin dilution was stored in the containers for one day.

used was difficult because of the variation in size and shape of the containers. It is striking, however, that the tuberculin from the 500 ml. Erlenmeyer flasks filled to capacity seemed to be stronger than that from any of the other containers, and that the "volume effect" was very small for this type of container. Since the 500 ml. Erlenmeyer flasks presented less surface per unit volume of contents than the other containers, this result might indicate that the loss of activity was connected with some property of the surface of the container. However, this assumption was not supported by the fact that the "volume effect" was observed in containers of materials with widely different chemical and physical properties.

Experiments designed to identify the causal factor: The inactivation described in the foregoing might be due to some factor associated with the air-filled space in the container. It is conceivable, for example, that the oxygen in the air affects the tuberculin or that the surface tension at the air-liquid interface causes denaturation of the tuberculoprotein.

However, as mentioned above, the inactivation of tuberculin might equally well be due to some effect of the container. Accordingly, a number of experiments were made to investigate these possibilities.

Denaturation at the air-liquid interface has been suggested as a cause of inactivation of proteins.[4] This form of inactivation, it must be assumed, increases as the ratio of surface to volume of solution increases and it might therefore explain the "volume effect."

Adams[4] tested the hypothesis of surface denaturation by experiments in which he increased the surface of the solution by shaking.

[4] ADAMS, M. H.: J. Gen. Physiol., 1947–1948, *31*, 417.

TABLE 4

MEAN SIZE OF REACTIONS (MM.)* IN BCG-VACCINATED GUINEA PIGS TO INTRADERMAL TESTS WITH 5 TU DILUTIONS STORED IN AMPOULES WITH AND WITHOUT GLASS FRAGMENTS†

Volume of 5 TU Dilution	Volume of Glass Fragments	Volume of Air	Mean
ml.	*ml.*	*ml.*	*mm.*
20	0	3	10.2 10.7
10	0	13	10.2 10.4
10	10	3	4.1 4.0

* Each mean refers to one ampoule and is based on 8 tests.

† The tuberculin dilution was stored in the ampoules for eight days.

In the present experiments, with tuberculin dilutions, there was no indication of a surface denaturation, although shaking did have an effect. In some cases the potency was increased after shaking. This phenomenon will be studied further.

Exclusion of air from the partially filled ampoules was in some experiments effected by adding paraffin oil. The loss of potency was, however, unaffected by the addition of paraffin oil.

The effect of contact with glass was examined by putting glass fragments in the tuberculin dilution. Ampoules of the kind used in these experiments were broken and the fragments sorted by sedimentation in water. The fragments used were fairly uniform in size, measuring from a few millimeters to not much less than a millimeter. A number of ordinary 20 ml. ampoules were filled with such fragments and tuberculin dilution was added till almost no air was left. The volume of dilution in these ampoules was approximately 10 ml. At the same time other ampoules were filled with 20 ml. and 10 ml. of tuberculin dilution, leaving an air volume of 3 and 13 ml., respectively.

The results (table 4) showed marked inactivation of tuberculin dilution in the ampoules containing glass fragments and no perceptible inactivation in the ampoules with an equal volume of air. From this it would seem that it is the glass and not the air which is of significance.

Further experiments were therefore made to find which property of the glass is responsible for the inactivation of tuberculin.

Metal ions are known to damage many biologic products, and the release of metal ions from the glass might therefore possibly explain the "volume effect." To test this hypothesis, use was made of certain compounds, so-called chelating agents which form stable complexes with metal ions. In some of the experiments described in the foregoing, chinosol, which is a chelating agent, was added to the diluent because of its antiseptic properties. It did not prevent the "volume effect." Furthermore, in two experiments comparisons were made between tuberculin dilutions prepared with chinosol (1 per ml.) or another chelating agent, Tetracemin[5] (ethylen-diamin-tetraacetic acid—10 per ml.), and tuberculin dilutions prepared without a chelating agent; there was no demonstrable difference in "volume effect."

[5] The Tetracemin® was kindly supplied by Ferrosan, Copenhagen.

TABLE 5

MEAN SIZE OF REACTIONS (MM.) IN BCG-VACCINATED GUINEA PIGS TO INTRADERMAL TESTS WITH BUFFER CONTAINING 0.20/00 TWEEN 80, STORED IN AMPOULES PREVIOUSLY CONTAINING 5, 25, AND 125 TU DILUTIONS COMPARED TO CONTROL 1 AND 5 TU DILUTIONS

Ampoules Previously Containing Tuberculin Dilutions of Strength*			Control Dilutions of Strength†	
5 TU	25 TU	125 TU	1 TU	5 TU
7.9	11.6	11.4		
8.7	8.1	11.1	4.6	8.5
7.2	10.7	8.4		

* Each mean refers to one ampoule and is based on 3 tests.

† Each mean is based on 7 tests.

The alkalizing effect often shown by glass might be of significance. To check this point pH determinations were made on the contents of a sample of ampoules used in these experiments. No difference in pH was demonstrated between the contents of completely and partially filled ampoules. It was found to be very close to 7.36, the expected pH of the buffered diluent.

Adsorption to glass of tuberculin was demonstrated by Parish[6] and has recently been discussed by Bretey.[7] As mentioned in the previous communication,[1] some of the early experiments also indicated that such adsorption takes place. In those experiments attempts were made to saturate the inner surface of the ampoules by pre-washing with tuberculin dilution. The results sometimes showed a difference in potency between dilutions stored in "saturated" and "unsaturated" ampoules. Similar trials with the various containers described in the foregoing showed the same trend. The results were, however, rather inconsistent and, although the "volume effect" was often reduced, it was never eliminated by the attempts to saturate the glass.

It is known from tests with another protein (testicular hyaluronidase) that glass which has been in contact with protein solution yields relatively large amounts of protein when eluted with appropriate buffers.[8] In early experiments of this series attempts were made to elute tuberculin from ampoules which had contained tuberculin dilutions by filling them with standard diluent. The results were negative, the diluent showing no biologic activity.

Later, when it was found that the addition of Tween 80 to tuberculin dilutions suppressed the "volume effect," the elution experiments were repeated with diluent containing Tween 80. Twenty milliliter ampoules containing 5, 25, or 125 TU dilutions were emptied and rinsed three times with standard diluent. The ampoules were then filled with 10 ml. of diluent containing 0.2 per ml. of Tween 80 and allowed to stand for five days, after which time the contents were tested for biologic activity.

As shown in table 5, tuberculin was eluted from all ampoules. The eluate from ampoules previously filled with 25 TU or 125 TU dilutions gave, on the average, stronger reactions than the control 5 TU dilution, while the contents of ampoules previously filled with 5 TU dilution elicited weaker reactions.

That tuberculin can be eluted from ampoules having previously contained tuberculin

[6] PARISH, H. J., AND O'BRIEN, R. A.: Brit. M. J., 1935, No. 3880, 1018.

[7] BRETEY, J.: Rev. de la tuberc., 1955, *19*, 427.

[8] STRØIER RASMUSSEN, P.: Acta chem Scandinav., 1954, *8*, 633.

has been confirmed in later experiments. In these it was also found that, relatively, more tuberculin could be eluted from partially filled than from completely filled ampoules. This finding seems to explain the "volume effect." However, details of these experiments will not be given at present, as the adsorption and subsequent elution of tuberculin seem to vary with as yet unidentified factors.

In summary: The results of further research into the instability of dilutions of purified tuberculin are reported. In early studies it was found that the instability did not appear as a progressive reduction in the potency of the dilution but as unsystematic variations between ampoules filled with the same dilution. Further investigation of the "inter-ampoule variation" revealed another phenomenon: tuberculin from partially filled ampoules was consistently less potent than tuberculin from completely filled ampoules. This phenomenon—the "volume effect"—is assumed to be due to the same cause as the "inter-ampoule variation" and, since it is more easily demonstrated, its occurrence under various conditions has been studied.

The "volume effect" occurs with different, commonly used preparations of purified tuberculin, although in varying degree. It cannot be prevented by using silicone-coated ampoules or by using containers of other materials, such as quartz or nylon. It is reduced, however, by the addition to the diluent of gelatine, gum arabic, or Tween 80.

Oxidation or denaturation at the air-liquid interface does not seem to be of significance, nor does inactivation by metal ions.

It has been demonstrated that tuberculin can be adsorbed to glass, in that tuberculin has been eluted from ampoules with a diluent containing Tween 80. It is therefore suggested that the "volume effect" is due to adsorption to the surface of the container.

World Health Organization
Tuberculosis Research Office
Copenhagen, Denmark

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July 21, 1955

[a] The writers are indebted to Dr. Florence Seibert of the Henry Phipps Institute, Philadelphia, for supplying SOTT-4; and to Dr. A. B. Paterson of the Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, for supplying freeze-dried PPD. Special thanks are due to Dr. Knud Tolderlund, Statens Seruminstitut, Copenhagen, for his valuable support and great interest in the work.

Books

HEURES INTERNATIONALES DANS LA LUTTE CONTRE LA TUBERCULOSE. By Étienne Bernard, pp. 94, Masson et Cie, Paris, 1955. Price, 950 fr.

This book contains a series of speeches delivered by the author during the years 1950 to 1955 at meetings of the International Union Against Tuberculosis, held in Copenhagen, Rio de Janeiro, and Madrid, at meetings celebrating the fiftieth anniversary of the tuberculosis associations of Belgium, Switzerland, and the United States, and at a meeting in French North Africa.

The author reviews in these talks the history of the fight against tuberculosis, with special emphasis on the contributions of the various countries he has visited and on the role the national tuberculosis associations and the International Union Against Tuberculosis are fulfilling in carrying on this campaign. He surveys the present status of our means and methods to prevent and treat tuberculosis and insists on early detection by systematic roentgenographic examinations and immediate treatment. This should be initiated in great specialized urban centers equipped with all diagnostic and therapeutic facilities including specialized laboratories in which drug-resistance tests and studies on cardiopulmonary function can be performed.

The author insists on flexibility and adaptability of our antituberculous equipment to the changing aspect of the disease. He especially singles out certain categories of patients who do not fit into the general scheme of the classical tuberculosis hospital. These categories include patients with minimal lesions, convalescents from tuberculous meningitis and miliary tuberculosis, and the old chronic cases. A program of rehabilitation and prevention of relapse should be part of the medical care for tuberculous patients. The author stresses the importance of improvement of living conditions and BCG vaccination, especially for highly exposed groups of the population and as the principal measure for the underdeveloped countries.

This book is written in a pleasant style and will be profitable to all who wish to obtain a lucid orientation in the broad medico-social aspects of the world-wide problem of tuberculosis.

WERNER B. SCHAEFER
DENVER, COLORADO

DISEASES OF THE CHEST. By H. Corwin Hinshaw and L. Henry Garland. Pp. 727, 288 illustrations, W. B. Saunders Company, Philadelphia, 1956. Price, $15.00.

This is a well-constructed book, notable for the extent and thoroughness of its coverage. The reviewer's first impression of a practical and trustworthy account of pulmonary disease in broad aspect was sustained by careful, detailed reading. The volume should be valuable to the general practitioner and specialist alike as a text and reference work for all the major pulmonary illnesses. It is written with a smooth and flowing style, free from evidence of disjointed authorship, remarkable for its space-saving facility. Without the latter the wide coverage could not have been accomplished; even in the shortest sections the reader is not left with the feeling that the subject has been slighted.

There are forty chapters, the first four of which are devoted to diagnostic procedures. The combination of authorship—internist and radiologist—is particularly effective here. The next two chapters deal with basic anatomy and physiology. The compilation then

proceeds with an account of the acute pulmonary inflammations, the mechanical embarrassments of respiration, neoplastic disease, circulatory abnormalities of the lungs, pulmonary tuberculosis, sarcoidosis, mycotic and parasitic disease, and pulmonary disorders of occupational origin. Succinct tables of contents prefacing each chapter orient the reader helpfully. Three of the chapters, on pulmonary physiology, foreign bodies in the larynx and bronchi, and coccidioidomycosis, were written by colleagues of the authors, viz., Roger H. L. Wilson, Walter E. Heck, and William A. Winn. In every chapter attention is paid to etiology and pathogenesis, as well as diagnosis and treatment.

Most readers will probably agree with the reviewer that the presentation is authoritative without being opinionated. In the tuberculosis chapters, in the light of the book's authorship, one might expect an overbalance in the space devoted to antimicrobial therapy. Actually the older methods are accorded quite as much room. Collapse therapy is introduced with the comment that readjustment of opinion in the light of the newer therapy has not yet become stabilized, but has resulted in a more critical attitude toward all conventional methods of treatment.

The illustrations, the majority of which are reproductions of roentgenograms, are of high caliber, and the press work is excellent.

Esmond R. Long
Pedlar Mills, Virginia

Pathology Seminars. Edited by Robert S. Haukohl and W. A. D. Anderson. Pp. 195. The C. V. Mosby Company, St. Louis, Missouri, 1955. Price, $10.00.

Most pathologists would welcome the opportunity to attend seminars moderated by the five well-known and highly respected contributors to this book, in which emphasis is given to the cytologic criteria for the diagnoses of a wide variety of neoplastic lesions by means of well-chosen case reports and series of pertinent questions and answers.

By nature the slide seminar is informal. This is manifest in the text in a very free expression of opinion by the discussants, recorded in a pleasant literary style. During the discussions a clear distinction is not always made between statements that are based primarily upon personal opinion and those which are derived from well-established facts. Since this is often left to the reader, with an inadequate bibliography, the value of the text is limited as a reference source for students of pathology. Nevertheless, the book offers a very enjoyable, informative, and stimulating experience to pathologists and oncologists that is secondary only to attendance at the original slide seminars.

F. Stephen Vogel
New York, New York

BOOKS RECEIVED

A Statistical Program for the Department of Health of the City of New York. By the Committee on Statistical Program for the City of New York. Pp. 71, Russell Sage Foundation, New York, 1956. Price, $1.00.

Il Pneumotorace Intrapleurico: Ieri ed Oggi. By Giusto Fegiz and Michele Lucchesi. Pp. 164, Edizione Mediche E Scientifiche, Rome, 1955.

Annual Review of Medicine. Edited by David A. Rytand. Pp. 611, Annual Reviews, Inc., Stanford, California, 1956. Price, $7.00.

Tuberculosis in Obstetrics and Gynecology. By George Schaefer. Pp. 307, Little, Brown and Company, Boston, 1956. Price $8.75.

Recherche, Isolement et Etude du Bacille Tuberculeux. By Fernand Tison and Jean Audrin. Pp. 104, Masson & Cie, Paris, 1956. Price, 1,000 fr.

CIBA FOUNDATION SYMPOSIUM ON HISTAMINE. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 472, Little, Brown and Company, Boston, 1956. Price, $9.00.

CONFERENCES D'ACTUALITES PRATIQUES: COEUR ET POUMONS. By P. Soulie *et al.* Pp. 224, Masson & Cie, Paris, 1956. Price, 1,600 fr.

CONFERENCES D'ACTUALITES PRATIQUES: MAL DE POTT. By S. De Sèze and J. Debeyre. Pp. 102, Masson & Cie, Paris, 1956. Price, 900 fr.

SOCIAL-MEDICAL INVESTIGATIONS ON TUBERCULOSIS IN THE COUNTY OF HORDALAND. By Knut Engedal. Pp. 126, A. S. John Griegs Boktrykkeri, Bergen, 1956.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

NEW YORK MEDICAL COLLEGE—FLOWER AND FIFTH AVENUE HOSPITALS

Postgraduate Course in Pediatric Allergy

A postgraduate course in pediatric allergy will be given at New York Medical College-Flower and Fifth Avenue Hospitals, Division of Graduate Studies, Department of Graduate Pediatrics, under the direction of Bret Ratner, M.D., Professor of Clinical Pediatrics and Associate Professor of Immunology, from November 7, 1956, to May 29, 1957.

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FIRST INTER-AMERICAN CONFERENCE ON OCCUPATIONAL MEDICINE AND TOXICOLOGY

The University of Miami School of Medicine and the University of Havana, Cuba, School of Medicine will hold jointly the first Inter-American Conference on Occupational Medicine and Toxicology in Miami, Florida, from September 3 to 7, 1956.

NINTH ANNUAL SYMPOSIUM ON PULMONARY DISEASES

Fitzsimons Army Hospital, Denver, Colorado

The Ninth Annual Symposium on Pulmonary Diseases will be held at Fitzsimons Army Hospital, Denver, Colorado, September 10–14, 1956. This Symposium will be co-sponsored by the American Trudeau Society, the University of Colorado School of Medicine, and Fitzsimons Army Hospital.

Information concerning enrollment may be obtained from the University of Colorado Medical School or from the Program Director, Colonel James A. Wier, Fitzsimons Army Hospital, Denver 8, Colorado.

FORTHCOMING ANNUAL SESSIONS OF THE AMERICAN COLLEGE OF PHYSICIANS

1957—38th Annual Session, Boston, Mass., April 8–12.

Richard P. Stetson, M.D., F.A.C.P., 203 Commonwealth Ave., Boston 16, Mass., General Chairman.

Walter L. Palmer, M.D., F.A.C.P., University of Chicago Department of Medicine, Chicago 37, Ill., President.

Edward R. Loveland, 4200 Pine St., Philadelphia 4, Pa., Executive Secretary and General Manager.

1958—39th Annual Session, Atlantic City, N. J., April 28–May 2.

James F. Gleason, M.D., F.A.C.P., 7 S. Oxford Ave., Atlantic City, N. J., General Chairman.

THE AMERICAN COLLEGE OF PHYSICIANS

Schedule of Postgraduate Courses, Autumn, 1956

The following courses have been arranged through the generous cooperation of the directors and the institutions at which the courses will be given. Tuition fees for all courses

will be: Members, $30.00; Non-members, $60.00. Full details of these courses may be obtained through the Executive Offices of the College, 4200 Pine Street, Philadelphia 4, Pa.

Course No. 1, Recent Advances in Cardiovascular Disease: Mt. Sinai Hospital, New York, N. Y.; Arthur M. Master, M.D., F.A.C.P., and Charles K. Friedberg, M.D., F.A.C.P., Co-directors. *October 8–12*

Course No. 2, Clinical Neurology: Jefferson Medical College of Philadelphia, Philadelphia, Pa.; Bernard J. Alpers, M.D., F.A.C.P., Director *October 15–19*

Course No. 3, Internal Medicine: Vanderbilt University School of Medicine, Nashville, Tenn.; Rudolph H. Kampmeier, M.D., F.A.C.P., and Hugh J. Morgan, M.D., M.A.C.P., Co-directors. *October 15–19*

Course No. 4, Recent Advances in Internal Medicine: University of Washington School of Medicine, Seattle, Wash.; Robert H. Williams, M.D., F.A.C.P., Director. *October 22–26*

Course No. 5, Selected Problems in Internal Medicine: University of Oklahoma School of Medicine, Oklahoma City, Okla.; Stewart G. Wolf, Jr., M.D., F.A.C.P., Director. *November 26–30*

Course No. 6, Gastro-Enterology: University of Pennsylvania Graduate School of Medicine, Philadelphia, Pa.; Henry L. Bockus, M.D., F.A.C.P., Director. *December 3–7*

Course No. 7, Electrocardiography: University of Utah College of Medicine and Salt Lake County General Hospital, Salt Lake City, Utah; Hans H. Hecht, M.D., Director. *December 3–8*

Course No. 8, Pathologic Physiology of the Blood Dyscrasias: Washington University School of Medicine, St. Louis, Mo.; Carl V. Moore, M.D., F.A.C.P., William J. Harrington, M.D. (Associate), and Edward H. Reinhard, M.D. (Associate), Co-directors. *February 18–22*

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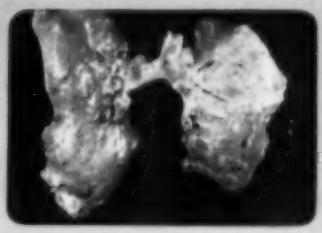

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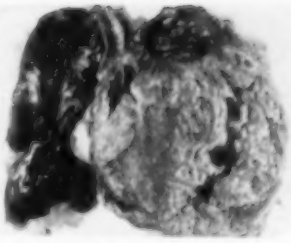

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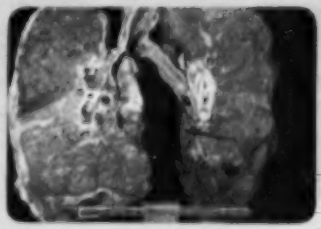

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1. *Molthan, L., Cohen, B. V., and Zarafonetis, C. J. D.: Clinical use of potassium para-aminosalicylate (KPAS). Am. Rev. of Tuberc. and Pulmon. Dis. 71:290 (Feb.) 1955.*

2. *Cohen, B. V., Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations). Read before the American Trudeau Society, Annual Meeting, Milwaukee, Wisconsin, May 23-27, 1955.*

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